The great tragedy of science -- the slaying of a beautiful hypothesis by an ugly fact.

- Thomas Huxley

University of Alberta

The role of 5-HT_{1B} and 5-HT_{2C} receptor ligands in reinforcement

by

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©

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Abstract

Within the brain, 5-hydroxytrytamine (5-HT) may play a role in reward and reinforcement, possibly through regulation of the mesocorticolimbic dopamine (DA) system. This thesis examined the effects of 5-HT_{1B} and 5-HT_{2C} receptor ligands on reinforcement by measuring place conditioning, conditioned taste aversion (CTA) learning and spontaneous locomotor activity, including nicotine-induced locomotor hyperactivity, to measure putative 5-HT/DA interactions, in rats. Drugs were administered to male Sprague-Dawley rats systemically or by microinjection into the ventral tegmental area (VTA). Place conditioning effects were assessed using a two-compartment place conditioning apparatus; CTA was assessed using a two-bottle choice test. Effects on spontaneous locomotor activity were monitored using photocell locomotor activity boxes. The main findings from these experiments were: (1) systemic administration of 5-HT_{2C} receptor agonists may decrease spontaneous locomotor activity; (2) 5-HT_{2C} receptor stimulation may only induce place conditioning effects when animals are tested in a drugged state; 5-HT_{2C} receptor activation may also induce a CTA; (3) stimulation of the 5-HT_{1B} receptor may not effect spontaneous locomotor activity, but, activation of this receptor may induce a conditioned place aversion when animals are tested in a drug-free state; (4) systemic, but not intra-VTA, administration of the 5-HT_{2C} receptor agonist WAY 161503 may decrease spontaneous and nicotine-induced locomotor activity; and (5) systemic but not intra-VTA administration of the 5-HT_{1B} receptor agonist CP 94253 may increase nicotine-induced hyperactivity. These results indicate that stimulation of the 5-HT_{1B} receptor may not alter locomotor activity alone, but may enhance the psychomotor stimulant effects of nicotine. In contrast, 5-HT_{2C}

receptor activation may decrease spontaneous and nicotine-induced locomotor activity; however this effect may not be mediated by an action at the VTA. 5-HT_{2C} or 5-HT_{1B} receptor stimulation may induce aversive reinforcing effects, but, in contrast to 5-HT_{1B} receptor activation, 5-HT_{2C} receptor activation may only induce place conditioning effects when animals are tested in a drugged state, possibly due to effects on the animals' perception of the environment. These behavioural experiments indicate that stimulation of the 5-HT_{1B} or 5-HT_{2C} receptor may have similar effects on behavioural tests of reinforcement; however, stimulation of these receptors may exert opposing effects on mesocorticolimbic DA as measured by alterations in nicotine-induced hyperactivity.

For Mom & Dad

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List of Abbreviations, Symbols and Nomenclature

°	degree(s)
5-HT	5-hydroxytryptamine; serotonin
5-HTP	5-hydroxytryptophan
5-HIAA	5-hydroxyindoleacetic acid
8-OHDPAT	8-hydroxy-2-(di-n-propylamin)tetralin
aCSF	artificial cerebrospinal fluid
AMGY	amygdala
AMPH	α-methylphenylethylamine; amphetamine
ANOVA	analysis of variance
AP	anterior-posterior
APo	area posterma
BG	basal ganglia
C	cortex
Ca^{+2}	calcium ion
cAMP	cyclic adenosine monophosphate
CB	cerebellum
CD	caudate putamen
cm	centimeter(s)
CGS 12066B	7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline
Co	colliculi
COMT	catechol-O-methyltransferase
CP	choroid plexus

CP 93129	1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*- pyrrolo[3,2-*b*]pyridine-5-one
CP 94253	5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine
CPA	conditioned place aversion
CPP	conditioned place preference
CSF	cerebrospinal fluid
CTA	conditioned taste aversion
DA	dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphé nucleus
DV	dorsal-ventral
EC	entorhinal cortex
FCX	frontal cortex
FMN	facial motor neurons
g	gram(s)
GABA	γ-aminobutyric acid
GLU	glutamate
GP	globus palldius
GR 127935	N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide
H	hippocampus
hr	hour(s)
HT	hypothalamus

HVA	homovanillic acid
I	ileum
IC	islands of Calleja
ICSS	intracranial self-stimulation
i.p.	intraperitoneal
K^+	potassium ion
kg	kilogram(s)
L	limbic system
L-DOPA	L-3,4-dihydroxyphenylalanine
LM	lateral-medial
m	meter(s)
MAO	monoamine oxidase
mCPP	1-(m-chlorophenyl)piperazine
Me	medulla
mg	milligram(s)
min	minute(s)
MK 212	6-chloro-2-(1-piperazinyl)pyrazine
ml	milliliter(s)
mm	millimeters(s)
mM	millimolar
mRNA	messenger ribonucleic acid
MRN	medial raphé nucleus
Na^+	sodium ion

nAch	nicotinic acetylcholine
NAD	nicotinamide adenine dinucleotide
NAS	nucleus accumbens
OB	olfactory bulbs
OT	olfactory tubercle
PBS	phosphate-buffered saline
PET	positron emission tomography
PFC	prefrontal cortex
PN	peripheral neurons
RN	raphé nucleus
Ro 60-0175	(S)-2-(chloro-5-fluoro-indol-l-yl)-1-methylethylamine
RS 102221	8-(5-(5-amino-2,4-dimethoxyphenyl)-5-oxopentyl)-1,3,8-triazaspiro(4.5)decane-2,4-dione
RU 24969	5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole hemisuccinate
S	striatum
SB 242084	6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline
s.c.	subcutaneous
SDZ SER082	(+)-*cis*-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methyindolol[1,7-bc] [2,6]-naftiridine fumarate
sec	second(s)
SN	substantia nigra
T	thalamus

TFMPP	N-[3-(trifluoromethyl)phenyl] piperazine
µg	microgram(s)
µL	microliter(s)
µm	micrometer(s)
veh	vehicle
VP	ventral pallidum
VTA	ventral tegmental area
WAY 100135	(S)-N-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide
WAY 100635	N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl-cyclohexanecarboxamine
WAY 161503	8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride

Chapter 1. Introduction

Examining the neural organization of behaviour is a major area of research in neuroscience/psychology (Hebb, 1949). In an attempt to learn why organisms perform certain behaviours over others, much research has focused on investigating the neural basis of motivation and reward. Experiments with laboratory animals in this field have contributed to our knowledge related to the neural circuitry underlying psychiatric disorders that display impairments in motivation and reward. For reviews on animal models of psychiatric disorders see Geyer and Markou (1995), Geyer and Moghaddam (2002), McKinney (1974), McKinney and Bunney (1969) and Willner (1984).

Reward is operationally defined by approach behaviour and is used to indicate the idea of pleasure. Therefore, rewarding stimuli are those "objects or events that elicit approach and are worked for (Wise, 2004)." In contrast, aversive stimuli are those which are avoided upon subsequent presentation (Beck, 1978). Reinforcement has been described by Thorndike in his "law of effect". The "law of effect" suggests that the outcome of a behaviour will determine if that behaviour is repeated (Beck, 1978; Bolles, 1967). In addition, Pavlov described reinforcement as the associative learning that occurs between unconditioned and conditioned stimuli (Wise, 1999). Thus, reinforcement may be defined as the strengthening of stimulus-response (Thorndike) or stimulus-stimulus (Pavlov) associations following the presentation of a particular event or reward (Wise, 2004). In this thesis, the terms 'reward' and 'reinforcement' are used interchangeably.

Motivation is a concept used to describe factors which cause an organism to engage in and maintain behaviour towards a goal/stimulus (Beck, 1978; Salamone and

Correa, 2002). Furthermore, Bindra (1974) suggested that motivational processes should be attributed to both the internal state of the organism and stimulus properties of objects in the environment. Incentive motivation (Bindra, 1978) refers to the idea that the anticipation of reinforcement can influence behaviour. Thus, the animal must have had prior exposure to the reinforcer and learned to predict the presentation of the reinforcer based on stimulus conditions (Bolles, 1967). Therefore, the animal may be more or less motivated to perform a behaviour based on previous experience with the reinforcer. Building on prior work, Bindra (1978) proposed that the properties of a stimulus which induce an organism to respond/react to it can be altered by the motivational state of the organism.

It should be noted that incentives differ from drives (drive theory was proposed by Hull, 1943). Incentive is learned though association with stimuli; drives are innate, unlearned mechanisms that result in behaviour directed towards drive reduction. As such, incentive motivation may occur in the presence of the proper stimulus conditions; drive changes depending on the present state of the organism. Finally, drive is generally considered an energetic phenomenon (e.g. it may 'push' the animal to do something). In contrast, incentive motivation is thought be an associative learning paradigm in which incentives 'pull' the animal towards a goal (Beck, 1978; Bolles, 1967). Thus, incentive motivation has directional properties which lead an organism towards a goal, rather than an innate mechanism that drives or pushes an organism towards drive reduction (an example would be drinking behaviour driven by thirst and leading to satiation of that drive).

Although the behavioural tests used in this thesis do not measure motivation, data obtained from behavioural studies examining the neural basis of reward/reinforcement may also be relevant to that of motivation. In laboratory animals, reinforcement can be studied using the drug self-administration, intracranial self-stimulation (ICSS) and place conditioning behavioural tests (these will be described below). In this thesis, the reinforcing effects of drugs were measured using the place conditioning behavioural test. Simply, place conditioning is a test in which animals are given access to two distinct neutral environments that are each paired with distinct unconditioned stimuli (e.g. drug or vehicle). When animals are later given free access to both conditioning environments, the time spent in each is used as an index of the reinforcing properties of the unconditioned stimulus, in this case – a drug state (Swerdlow et al., 1989). The place conditioning test was chosen as it is sensitive to both the positive and negative reinforcing effects of drugs and animals can be tested in a either a drug-free or drugged state (Bardo and Bevins, 2000; Swerdlow et al., 1989).

Compounds which act on 5-hydroxytryptamine (5-HT) receptor subtypes are active in behavioural tests used to measure reward and reinforcement. One of the primary neurotransmitters involved in reinforcement is dopamine (DA), and some studies suggest that the ability of 5-HT receptor ligands to alter reinforcement may be due to their effects on DA transmission within the mesocorticolimbic system. The goal of this thesis was to explore the effects of 5-HT receptor ligands which act at the 5-HT_{1B} and 5-HT_{2C} receptor on reinforcement. In this thesis, 5-HT_{1B} and 5-HT_{2C} receptor ligands were assessed in the place conditioning and spontaneous locomotor activity tests. In addition, nicotine-induced locomotor hyperactivity was used to assess DA/5-HT interactions following

administration of selective 5-HT_{1B} or 5-HT_{2C} receptor ligands either systemically or directly into the ventral tegmental area (VTA) of the brain. This chapter provides a brief review of 5-HT and DA receptor pharmacology, describes nicotine-induced hyperactivity and introduces the brain circuitry involved in reinforcement. A general description of the methodology used in this thesis is provided at the end of this chapter.

5-Hydroxytryptamine

5-HT is an indolealkylamine neurotransmitter that is synthesized in the brain from the amino acid L-tryptophan. L-tryptophan is converted to 5-hydroxytrytophan (5-HTP) via tryptophan hydroxylase; this is the rate-limiting step in 5-HT synthesis. 5-HT is then synthesized by the decarboxylation of 5-HTP by the enzyme L-aromatic amino acid decarboxylase (Figure 1.1). The action of 5-HT at the synapse is terminated by catabolism or by uptake of 5-HT into the nerve terminal by plasma membrane transporters. The catabolism of 5-HT is carried out by the enzymes monoamine oxidase (MAO) and aldehyde dehydrogenase. Depending on the ratio of NAD^+/NADH in the tissue, the intermediately metabolite 5-hydroxyindoleacetaldehyde is further broken down into the primary metabolite 5-hydroxyindoleacetic acid (5-HIAA) or 5-hydroxytryptophol respectively (Cooper et al., 2003; Feldman et al., 1997) (Figure 1.2).

Figure 1.1. Synthesis of 5-HT.

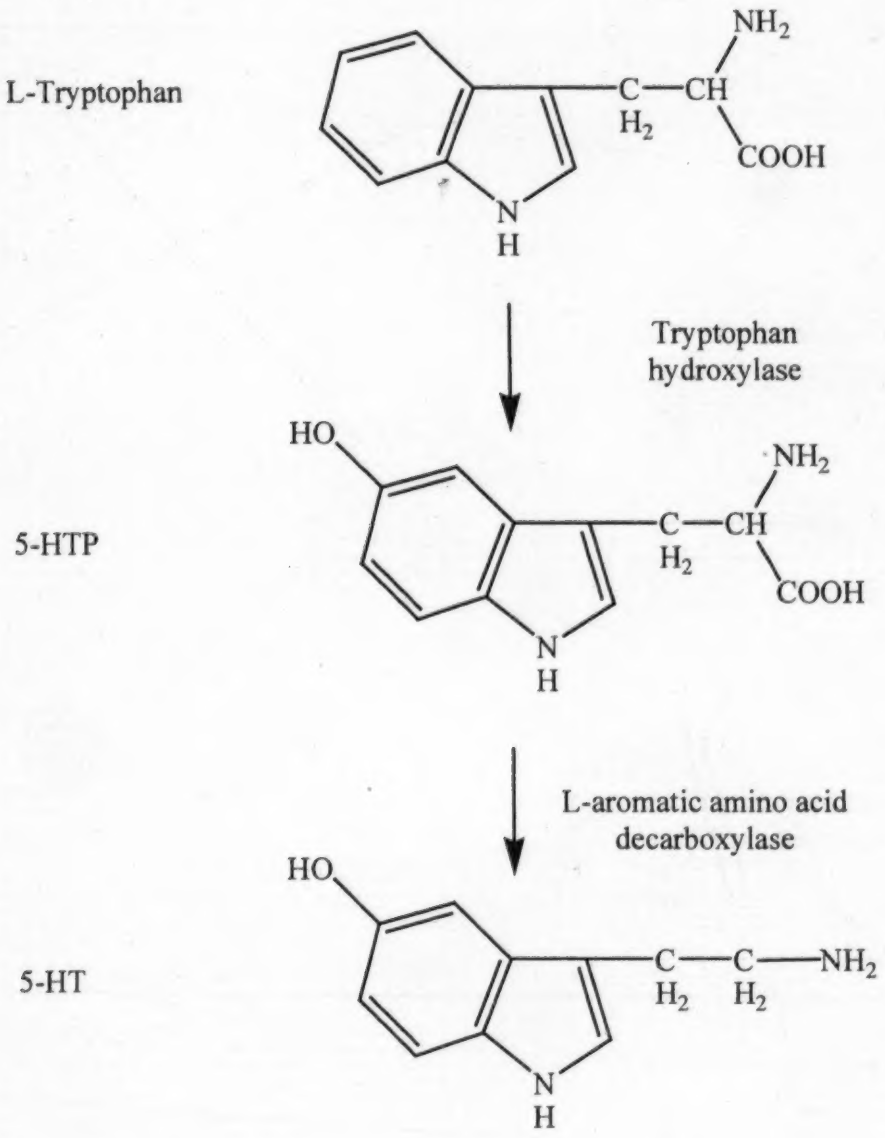
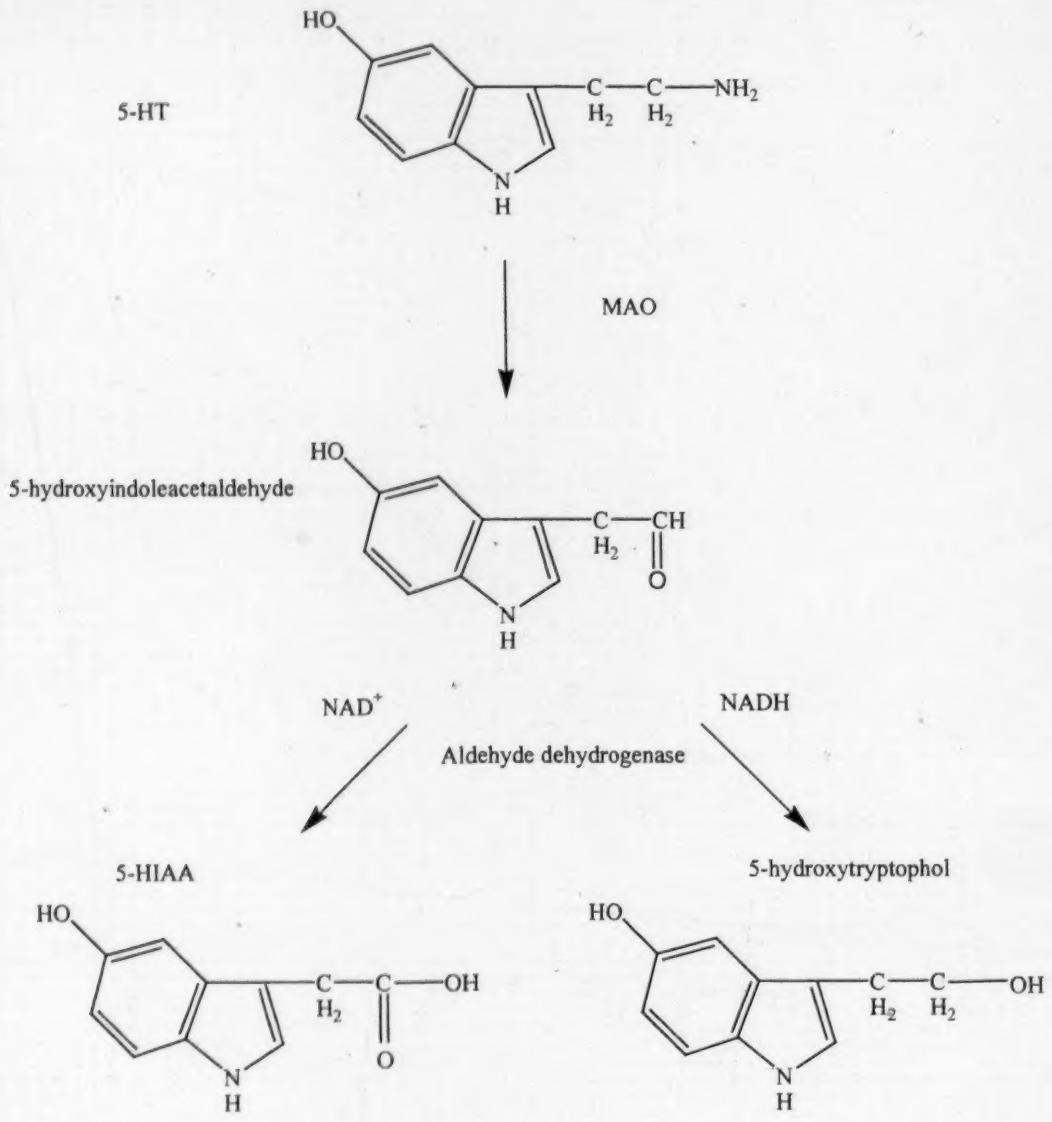


Figure 1.2. Metabolism of 5-HT.



Currently, seven families of 5-HT receptors are recognized by the Nomenclature Committee of the International Union of Pharmacology. Classification of receptor families is based on amino acid sequence homology, coupling to second messenger systems, signal transduction mechanisms, and receptor pharmacology. The 5-HT receptor families are classified as metabotropic (G-protein coupled) receptors with the exception of the 5-HT_3 receptor, which is a ligand-gated ion channel. 5-HT receptors that have been identified but currently have no known physiological role are designated as 5-ht receptors. The 5-HT_1 and 5-ht_5 receptor families are negatively coupled to adenylyl cyclase (and inhibit cyclic adenosine monophosphate (cAMP) formation) via an inhibitory G-protein. In contrast, the 5-HT_4, 5-ht_6 and 5-HT_7 receptor families positively modulate cAMP formation via a stimulatory G-protein coupled to adenylyl cyclase. The 5-HT_2 family of receptors acts to mobilize intra-cellular Ca^{+2} via hydrolysis of inositol phosphates as it is positively coupled via a G_q- protein to stimulate phospholipase C. In addition to the metabotropic 5-HT receptor families, the 5-HT_3 receptor is classified as a cation selective ligand-gated ion channel with equal permeability for K^+ and Na^+ (Table 1.1). For detailed reviews of the 5-HT receptor families and their function see Hoyer et al. (2002) and Barnes and Sharp (1999).

Table 1.1 Summary of 5-HT receptor subtypes. Modified from Cooper et al. (2003).

Family	Subtype	Effect	Agonist	Antagonist	Distribution
5-HT_1	1A	↓ cAMP	8-OHDPAT Buspirone BMY 7378	WAY 100635 WAY 100135	AMYG, OT, H, T, RN, C, L
	1B		CP 94253 CP 93129	GR 127935 GR 55562 SB 216641 SB 224289	SN, GP, VP, VTA
	1D		Sumatriptan GR 46611 L 694247	GR 127935 BRL 15572	SN, GP, BG
	5-ht_{1E}		BRL 54443		C, CD, AMYG
	5-ht_{1F}		BRL 54443		C, H, DRN
5-HT_2	2A	↑ IP_3/DAG	DOI DOB α-Me5-HT	MDL 100907 R 96544 SR 46349B	C, H, BG, FMN
	2B		BW 723C86	SB 204741	Stomach fundus, C
	2C		WAY 161503 WAY 629 Ro 60-0175 Ro 60-0332	SB 242084 RS 102221 4F 4PP SDZ SER-082	CP, Me, H, NAS, VTA
5-HT_3		Cation Channel	PBG SR 57227	Ondansetron MDL 72222 ICS 205-930 Y-25130 Zacopride LY 278584	APo, PN, EC
5-HT_4		↑ cAMP	SB 205149 BIMU8	GR113808 GR 125487 SB 204070A	H, Co, I
5-ht_5	5A	↓ cAMP			H, CB, C
	5B				DRN, H, OB
5-HT_6		↑ cAMP		SB 258510A SB 258585	S, NAS, C
5-HT_7		↑ cAMP	8-OHDPAT AS 19	SB 269970	T, HT, AMYG, Heart

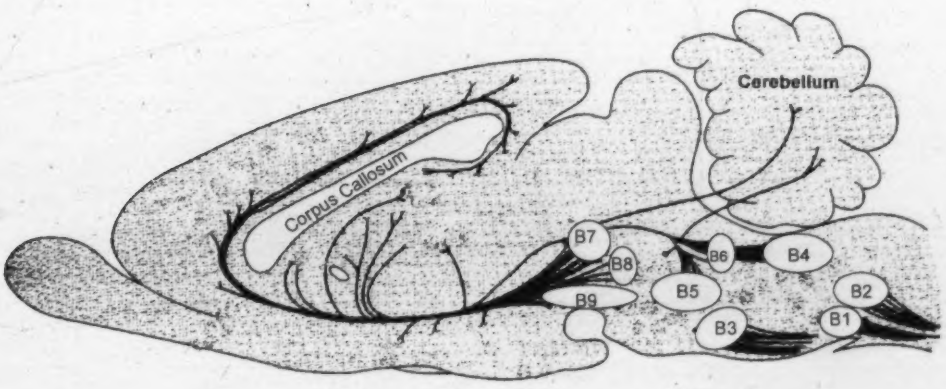
AMGY-amygdala; APo- area posterma; BG-basal ganglia; C-cortex; CB-cerebellum; CD- caudate putamen; Co-colliculi; CP-choroid plexus; DRN-dorsal raphé nucleus; EC-entorhinal cortex; FMN-facial motor neurons GP-globus pallidus; H-hippocampus; HT-hypothalamus; I-ileum; L-limbic system; Me-medulla; NAS-nucleus accumbens; OB-olfactory bulb; OT-olfactory tubercle; PN- peripheral neurons; RN-raphé nucleus; S-striatum; SN-substantia nigra; T-thalamus; VP-ventral pallidum; VTA-ventral tegmental area

5-HT receptors may be located on the terminals, soma or dendrites of presynaptic neurons (autoreceptors) or on post-synaptic neurons. Stimulation of autoreceptors located on the cell body or dendrites (5-HT_{1A} receptors) of the neuron may reduce neuronal cell firing thus inhibiting 5-HT release; stimulation of 5-HT autoreceptors located on the nerve terminal (5-$HT_{1B/D}$ receptors) inhibits the synthesis and release of 5-HT. In addition, 5-HT_{1B} receptors are also localized to the nerve terminal of other neuronal cell types (heteroceptors) such as such as glutamate, acetylcholine or γ-aminobutyric acid (GABA) where they inhibit the release of these neurotransmitters (Hoyer et al., 2002). The distribution of 5-HT receptors in the brain has been characterized using radioligand binding studies. A summary of the localization of 5-HT receptor subtypes as well as ligands for these receptors is provided in Table 1.1. The primary 5-HT receptor subtypes of interest in this thesis are the 5-HT_{1B} and 5-HT_{2C} receptors. The highest densities of 5-HT_{1B} receptors in the brain, are found in the dorsal subiculum, globus pallidus, ventral pallidum and the substantia nigra. Moderate densities of 5-HT_{1B} receptor binding sites are also found in the cerebral cortex, VTA, caudate nuclei, superior colliculus and hippocampus (Bruinvels et al., 1993; Pazos and Palacios, 1985; Sari, 2004). The 5-HT_{2C} receptor is widely distributed throughout the brain with the highest density of receptor binding sites consistently found in the choroid plexus; however moderate densities of this receptor are also present in the in the nucleus accumbens (NAS), VTA, olfactory nucleus, hippocampus, amygdala and the basal ganglia (Barnes and Sharp, 1999; Palacios et al., 1991; Pazos and Palacios, 1985; Pompeiano et al., 1994; Radja et al., 1991). The 5-HT_{1B} and 5-HT_{2C} receptor subtypes are of particular interest in the context of reward and reinforcement as they are located

within the mesocorticolimbic system a brain pathway implicated in reinforcement (Nestler, 2005; Wise and Rompre, 1989; Wise, 2002). Activation of these receptors may also alter DA release (the primary neurotransmitter implicated in reward/reinforcement) in terminal areas of this system. Regulation of DA function in the mesocorticolimbic system via action at 5-HT_{1B} or 5-HT_{2C} receptors will be discussed in more detail below.

Within the brain, the 5-HT systems originate from the midbrain raphé nuclei (B1-B9) located in the brainstem. The primary ascending projections of 5-HT neurons originate in the dorsal (DRN) and the median (MRN) raphé nucleus; together these projections account for 80 % of the 5-HT innervation to the forebrain (Azmitia and Segal, 1978). The DRN (B6/B7) and MRN (B5/B8) innervate many of the same target regions; however, the DRN primarily innervates the substantia nigra, thalamus, neostriatium as well as the cerebral and cerebellar cortices whereas the MRN projects largely to the limbic system (Cooper et al., 2003; Feldman et al., 1997). In addition to innervating different target areas, axonal projections from the DRN and MRN have distinct morphological characteristics. DRN neurons are identified by fine axons with small varicosities while axons with large varicosities (beaded axons) arise from the MRN (Kosofsky and Molliver, 1987). Projections from these midbrain raphé nuclei are shown in Figure 1.3.

Figure 1.3. Projections from the midbrain raphé nuclei. Modified from Saper (2000).



Dopamine

The neurotransmitter DA is synthesized from the amino acid L-tyrosine, which is obtained from dietary proteins. L-3,4-dihydroxyphenylalanine (L-DOPA) is the product of the conversion of L-tyrosine by the enzyme tyrosine hydroxylase; this is the rate-limiting step in DA synthesis. L-DOPA is then converted into DA by the enzyme L-aromatic amino acid decarboxylase (Figure 1.4). The action of DA at the synapse is terminated via reuptake into the nerve terminal and catabolism. DA can be converted into its primary metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by the enzymes MAO and catechol-O-methyltransferase (COMT) (Feldman et al., 1997) (Figure 1.5).

Figure 1.4. Synthesis of dopamine.

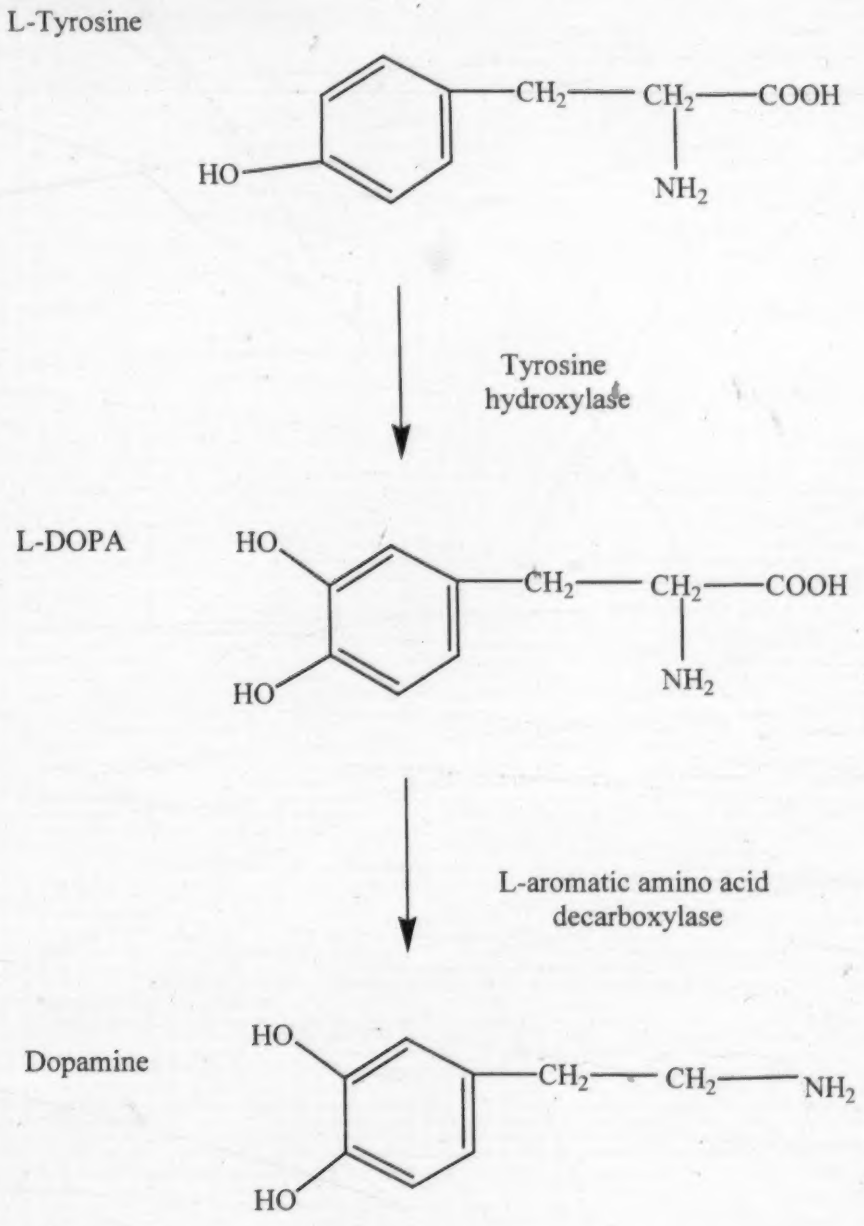
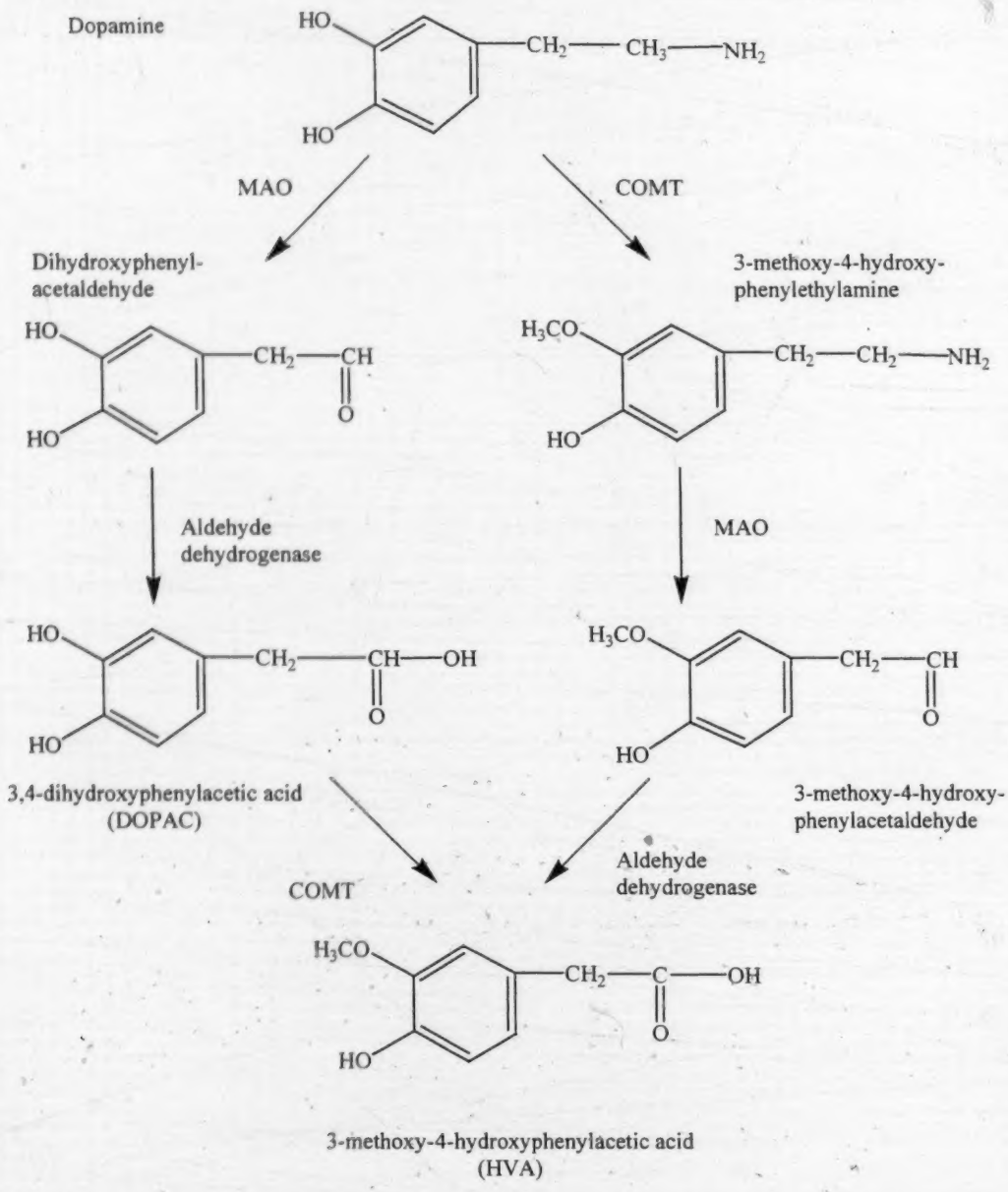


Figure 1.5. Metabolism of dopamine.



Currently, there are five families of DA receptors which are classified into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors based on the coupling of the receptor to adenylyl cyclase by a G-protein. D_1-like receptors are positively coupled to adenylyl cyclase and inhibit cAMP formation, while D_2-like receptors inhibit cAMP formation as they are negatively coupled to adenylyl cyclase (Table 1.2). DA receptors may be located on the soma, terminal or dendrites of DA neurons (autoreceptors) or on post-synaptic neurons. DA autoreceptors at believed to be mainly DA D_2 receptors (Starke et al., 1989); activation of DA autoreceptors located on the soma or dendrites acts to decrease cell firing, which may reduce DA release (Adell and Artigas, 2004), whereas activation of terminal autoreceptors inhibits the synthesis and release of DA (Starke et al., 1989).

Within the brain, there are four major DA systems: mesolimbic, mesocortical, nigrostriatial and the tuberoinfundibular systems. The primary function and the target sites for each of these systems are summarized in Table 1.3.

Table 1.2. Summary of dopamine receptor subtypes. Modified from Cooper et al. (2003).

Family	Subtype	Effect	Agonist	Antagonist	Distribution
D_1-like	D_1	↑ cAMP	SKF 38393	SCH 23390	CD, OT, NAS, AMYG, SN
	D_5				T, H, HT
D_2-like	D_2	↓ cAMP	Bromocriptine	Haloperidol Sulpiride	CD, OT, NAS, SN
	D_3		7-OHDPAT	UH 232	OT, NAS, IC
	D_4		CP 226269	Clozapine	FCX, AMYG, Midbrain

AMGY-amygdala; CD- caudate putamen; FCX-frontal cortex; H-hippocampus; HT-hypothalamus; IC-islands of Calleja; NAS-nucleus accumbens; OT-olfactory tubercle; SN-substantia nigra; T-thalamus

Table 1.3. Summary of central dopamine systems. Modified from Cooper et al. (2003).

System	Site of Origin	Termination Site(s)	Primary Function
Mesolimbic	VTA	NAS, OT	Reward & Reinforcement
Mesocortical	VTA	PFC, cingulate and entorhinal cortexes	Learning & Memory
Nigrostriatal	SN pars compacta	Dorsal striatum	Motor Function
Tuberoinfundibular	Periventricular and Arcuate Nuclei of the hypothalaus	Intermediate lobe of the pituitary and median eminence	Prolactin Release

Dopamine and 5-HT in Psychiatric Disorders

The focus of this thesis was to examine the effects of 5-HT receptor ligands in the context of reinforcement as well as interactions with DA in the mesocorticolimbic system. Dysfunction of these neurotransmitter systems appears to be a common aspect underlying the pathophysiology of many psychiatric disorders such as schizophrenia, depression, anxiety disorders as well as drug abuse. In addition, schizophrenia, depression and drug abuse also display impairments in motivation and reward. A brief overview of 5-HT and DA abnormalities reported in these disorders is given below.

The main neurochemical theories concerning schizophrenia, depression and drug abuse focus on abnormal functioning of brain monoamine neurotransmitter systems. For instance, the DA hypothesis of schizophrenia has remained the dominant theory used to explain the appearance of 'positive' (e.g. hallucinations and delusions) and 'negative' (e.g. anhedonia and alogia) symptoms seen in schizophrenic patients. This theory is based on the observation that positive symptoms of schizophrenia may be induced by increased DA levels in the limbic system whereas negative symptoms may be a result of reduced DA function in the prefrontal cortex (PFC) (Abi-Dargham, 2004; Abi-Dargham and Laruelle, 2005; Davis et al., 1991; Pickar et al., 1990). Support for the DA hypothesis has come from studies that demonstrate that psychostimulants such as amphetamine (AMPH), which acts as an indirect DA receptor agonist, can induce psychotic states (Connell, 1958; Randrup and Munkvad, 1967; Yui et al., 2000). For a review of DA and schizophrenia see Kapur and Mamo (2003). In addition to DA, 5-HT dysfunction has also been implicated in the pathophysiology of schizophrenia as action at 5-HT receptor subtypes may be responsible for the improvement of negative symptoms

observed following atypical antipsychotic drug treatment (for a review of 5-HT receptors and antipsychotic drug action see Meltzer, 1995; Meltzer et al., 2003). The monoamine hypothesis of depression suggests that this disorder may be caused by a decrease in monoamine neurotransmitters at important central synapses (Schildkraut, 1965). In addition to the pathophysiology of schizophrenia and depression, DA has also been implicated in the reinforcing properties of drugs of abuse. More specifically, Wise and Bozarth (1987) proposed that drugs of abuse such as cocaine, nicotine and AMPH may induce reinforcing effects through an increase in DA transmission in the NAS. This hypothesis was further supported by microdialysis studies that demonstrated that drugs of abuse increase both DA in the NAS as well as locomotor activity (Di Chiara and Imperato, 1988).

The primary metabolite of DA, HVA, has been found to be reduced in the venoarterial plasma concentrations of depressed patients (Lambert et al., 2000). In addition, the high incidence of depression among patients with Parkinson's disease also lends support for a role of DA in depression (Lemke et al., 2004). A decrease in either 5-HIAA (the primary metabolite of 5-HT) in the cerebrospinal fluid (CSF) or in urinary HVA levels has been correlated with increased risk of suicide in individuals who have previously attempted suicide (Nordstrom et al., 1994; Roy et al., 1992). Changes in 5-HT and DA receptor densities have also been observed in drug abusers as well as schizophrenic and depressed individuals. For instance, imaging studies using positron emission tomography (PET) revealed that schizophrenic patients and drugs abusers have an increased (Silvestri et al., 2000; Wong et al., 1986) and decreased (Volkow et al.,

1999; Volkow et al., 2001; Volkow et al., 2002) level of DA D_2 receptor binding sites respectively.

Depressed patients who were antidepressant-naïve displayed a higher incidence of 5-HT_{1A} receptor binding sites compared to control and medicated depressed patients (Parsey et al., 2006). Post-mortem studies on schizophrenic patients also found an increase in 5-HT_{1A} receptor density in the PFC (Burnet et al., 1996, 1997; Hashimoto et al., 1991); however this result was not confirmed by *in vivo* studies using PET (Bantick et al., 2004). In contrast to 5-HT_{1A} receptors, the density of 5-HT_{2A} receptors was decreased in the PFC and parahippocampal gryus in post mortem tissue taken from schizophrenic patients (Burnet et al., 1996; Dean et al., 1999); however, data from PET studies has yielded conflicting results (Lewis et al., 1999; Ngan et al., 2000; Okubo et al., 2000; Trichard et al., 1998). In addition, older, non-medicated depressed patients expressed reduced hippocampal 5-HT_{2A} receptor binding sites using PET (Sheline et al., 2004). For reviews on the role of 5-HT or DA in depression, see Dailly et al. (2004), Kapur and Mann (1992), Esposito (2006), Celada et al. (2004), Stockmeier (2003) and Elhwuegi (2004).

As DA and 5-HT dysfunction is believed to underlie the pathophysiology of schizophrenia, depression and drug abuse, therapeutic drug development used to treat these disorders has targeted central 5-HT and DA function. For example, 5-HT_{2C} receptor stimulation has been proposed as a potential treatment for drug abuse (Giorgetti and Tecott, 2004; Higgins and Fletcher, 2003) as activation of this receptor is known to decrease DA concentrations in the NAS (De Deurwaerdere et al., 2004; Di Matteo et al., 1999, 2000). A common mechanism of action of atypical antipsychotic drugs is blockade

of DA D_2 receptors (Creese et al., 1976; Seeman and Lee, 1975); however, the therapeutic effects reported following antipsychotic drug administration have been attributed to 5-HT_{1A} receptor stimulation, as well as blockade of 5-HT_2 receptors, (Bantick et al., 2001; Elliott and Reynolds, 1999; Jordan et al., 2002; Meltzer, 1995; Meltzer et al., 2003; Navailles et al., 2006; Reynolds, 2004). The drugs used to combat depression also include selective 5-HT reuptake inhibitors, 5-HT/noradrenaline reuptake inhibitors as well as MAO-inhibitors; the actions of these drugs are to increase monoamine neurotransmitter concentrations in the synapse (Cooper et al., 2003). This evidence indicates that alterations in central 5-HT and DA function are an important aspect underlying the pathophysiology of disorders which display impairments in motivation and reward.

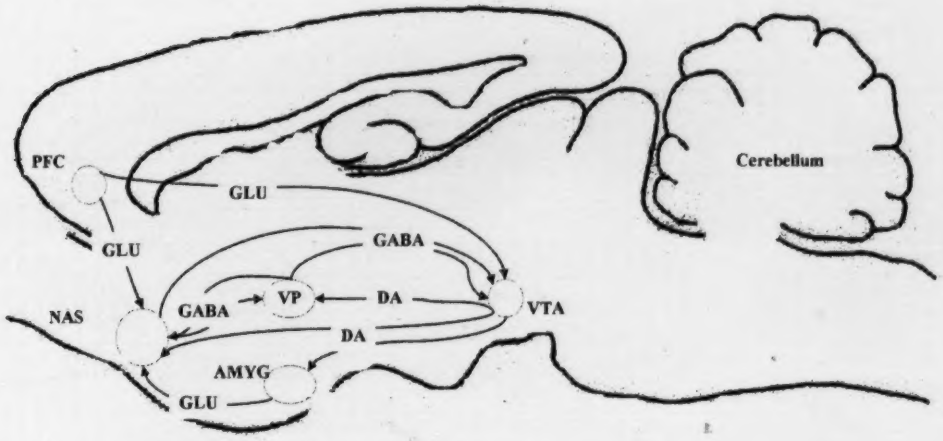
Brain circuitry of reward and reinforcement

The major brain neural pathway implicated in mediating reward and reinforcement is the mesocorticolimbic DA system. This system originates in the VTA which is located in the A10 region of the ventromedial mesencephalon and projects to the PFC and NAS (Dahlstrom and Fuxe, 1964; Wise and Rompre, 1989; Wise, 2002). The primary population of neurons within the VTA are dopaminergic, however, there is also subpopulation of non-tyrosine hydroxylase-containing neurons which are presumed to be GABA-containing (Kalivas, 1993; Nagai et al., 1983). The VTA primarily sends ascending DA projections to the NAS and PFC (Seroogy et al., 1989; Van Bockstaele and Pickel, 1995). In addition to ascending projections from the VTA, the NAS also receives descending glutamate input from the PFC. The VTA also receives descending glutamate

input from the PFC as well as GABA input from the NAS (Ikemoto and Panksepp, 1999; Taber and Fibiger, 1995).

In addition to connections between the NAS, VTA and PFC, the extended amygdala and ventral pallidum are also innervated by projections from the mesocorticolimbic system. The extended amygdala (which includes the central amygdala, NAS shell) receives DA input from the VTA and sends inhibitory GABA projections back to the VTA. In the context of Pavlovian conditioning, the amygdala may serve to retrieve emotional or motivational value of the unconditioned stimulus following exposure to the conditioned stimulus (Cardinal et al., 2002). The ventral pallidum is innervated by GABA neuronal projections from the NAS and DA projections from the VTA, and in turn, the ventral pallidum sends inhibitory GABA projections to both the NAS and VTA (Churchill and Kalivas, 1994). Although the role of the ventral pallidum is not well explored in the context of motivation and reward, it may be important due to its proposed role in transferring information between the basal ganglia (a motor related area) and the mesocorticolimbic system (Koob and Swerdlow, 1988). In addition, evidence from place conditioning studies indicates that the ventral pallidum may play a role in the acquisition of drug-reinforced behaviour (Kretschmer, 2000). Ascending and descending projections within the mesocorticolimbic system are depicted in Figure 1.6.

Figure 1.6. Ascending and descending neurotransmitter pathways in the mesocorticolimbic system. Adapted from Ikemoto and Panksepp (1999) and Kalivas and Volkow (2005).



In this thesis, the role of 5-HT_{1B} and 5-HT_{2C} receptors in the context of reinforcement was examined. Within the mesocorticolimbic system, 5-HT_{1B} and 5-HT_{2C} receptors are believed to be located on GABA containing neurons in the VTA (Bruinvels et al., 1994; Di Giovanni et al., 2001; Di Matteo et al., 2001; Di Matteo et al., 2002; Yan and Yan, 2001a). Stimulation of 5-HT_{2C} receptors localized to VTA GABA interneurons is hypothesized to decrease DA release in the VTA and NAS by increasing GABA inhibition onto DA neurons (Di Matteo et al., 2001). In contrast, activation of 5-HT_{1B} receptors decreases GABA release in the VTA as well as increase DA levels in both the VTA and NAS (O'Dell and Parsons, 2004; Yan and Yan, 2001a, b; Yan et al., 2004; Yan et al., 2005). As DA is known to play a role in reinforcement (Ikemoto and Panksepp,

1999; Ikemoto and Wise, 2004; Wise and Rompre, 1989; Wise, 2004), activation of 5-HT_{1B} or 5-HT_{2C} receptors may alter reinforcing behaviour via the modulation of DA levels in the mesocorticolimbic system. The 5-HT_{1B} and 5-HT_{2C} receptors were chosen as few studies have examined the effect on reinforcement following administration of selective ligands at these receptors. Although action at other 5-HT receptor subtypes can alter central DA activity, ligands at these receptors have been extensively examined in the context of reward (e.g. 5-HT_{1A}); data reported in studies using 5-HT_3 report ligands have been inconsistent and difficult to interpret. Furthermore, selective ligands for many 5-HT receptors, such as the 5-HT_{2A}, 5-HT_6 and 5-ht_5 receptors have not been developed; therefore, the effects these receptors play in reinforcement may be difficult to assess.

Role of mesocorticolimbic dopamine

It is well established that the neurotransmitter DA is involved in rewarding and reinforcing behaviours; however, the exact role DA plays in this context has been debated. Recently, mesocorticolimbic DA has been suggested to act as a signal encoding either the prediction error during reward-related learning or incentive salience to environmental cues rather than the reward itself (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; McClure et al., 2003; Schultz, 2002). There is emerging evidence from behavioural studies which support these proposed roles for mesocorticolimbic DA in reward/reinforcement. For instance, DA neuronal cell firing was found to increase or to decrease respectively following the presentation of conditioned stimuli to animals trained to discriminate between stimuli that predicted the presence or absence of a reward

(Tobler et al., 2003) or the magnitude of the reward (i.e. larger vs. smaller liquid volume) (Tobler et al., 2005). In agreement with the above studies, Nicola et al. (2004a; 2004b) demonstrated that neuronal cell firing patterns in the NAS were altered by reward-predicting cues; the observed pattern of neuronal cell firing may encode both information related to the prediction of reward (i.e. environmental cue) as well as the motor behaviour necessary to respond to the environmental cue (Nicola et al., 2004a, b). Furthermore, Wilson and Bowman (2005; 2006) reported that neurons in both the NAS and VTA may encode information related to the reward outcome predicted by the conditioned stimuli in rats trained to discriminate between an environmental cue which predicted a reward (i.e. delivery of saccharin) and that which predicted an aversion (i.e. delivery of quinine). VTA neuronal activity was also found to be inhibited during the consumption of a saccharin solution, indicating that changes in VTA neuronal activity may not be related to receiving the reward itself (Wilson and Bowman, 2006). Intra-NAS administration of the DA receptor antagonist SCH 23390 or the DA reuptake blocker GBR 12909 to rats trained on a task in which they were presented with either a predictive stimulus (i.e. animals had a 15% chance of receiving a reward) or a discriminative stimulus (i.e. animals had a 100% chance of receiving a reward) demonstrated that NAS DA is needed for proper responding to reward-predictive cues (Nicola et al., 2005). These studies indicate that mesocorticolimbic DA may be important in the context of learning evoked by reward-related cues.

5-HT$_{1B}$ and 5-HT$_{2C}$ receptors in reward and reinforcement

As the focus of this thesis was to investigate the role of 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors in reinforcement, a brief overview of studies examining the effects of ligands at these 5-HT receptor subtypes is presented below.

5-HT$_{1B}$ receptor-related ligands and place conditioning

Currently, only one study has reported the effects of selective 5-HT$_{1B}$ receptor activation using the place conditioning test. Cervo et al. (2002) found that the 5-HT$_{1B}$ receptor agonist CP 94253 dose-dependently induced a conditioning place aversion (CPA); this effect was blocked by pretreatment with the 5-HT$_{1B/D}$ receptor antagonist GR 127935. GR 127935 alone did not induce place conditioning. Further investigation revealed that CP 94253 potentiated a sub-threshold dose of cocaine to induce a conditioned place preference (CPP) but did not affect the CPP induced by higher doses of cocaine. GR 127935 reversed the CPP induced by the combination of CP 94253 and cocaine (Cervo et al., 2002). In general, it appears that activation of 5-HT$_{1B}$ receptors decreases reinforcement as indicated by the induction of a CPA, but may enhance the reinforcing effects of cocaine. As only one report has investigated the effects of 5-HT$_{1B}$ receptor ligands in place conditioning, further studies will be needed to verify the role of this receptor on conditioned reinforcement.

5-HT$_{1B}$ receptor-related ligands and intracranial self-stimulation

ICSS is a behavioural test which is used to determine brain regions that may be aversive or reinforcing following electrical stimulation to the area of interest. In addition, ICSS can be used to examine drug effects on reward. Rats, surgically implanted with an

electrode into the brain region of interest are trained to press a lever for the delivery of a reinforcing electrical stimulation (Olds and Milner, 1954). Some ICSS studies measure reinforcement based on response rates, however, data using this measure should be interpreted with caution as they may not be independent of performance effects (e.g. stimulant or sedative drug effects). The most common ICSS measure used is the rate-frequency curve-shift method. In this method, the electrical frequency that produces 50% of the maximal response rate is used as an index of reward. Additionally, potential performance effects induced by the test compound can be assessed by measuring the maximal response rates of each subject across treatments (Stellar and Rice, 1989; Wise, 1989). This measurement is known to be sensitive to changes in reinforcement induced by drugs such as drugs of abuse (Wise, 1996).

To date, no studies have reported the role of selective 5-HT_{1B} receptor ligands on ICSS, however there is evidence from Harrison et al. (1999) that stimulation of 5-HT_{1B} receptors may decrease reward in this behavioural test. The mixed 5-HT receptor agonist RU 24969 was found to increase ICSS current thresholds (indicating a decrease in reward) in animals implanted with bipolar lateral hypothalamic stimulating electrodes (Harrison et al., 1999). Blockade of 5-$HT_{1B/D}$ receptors by the antagonist GR 127935 did not affect ICSS current thresholds, but was able to prevent the threshold-elevating effect of RU 24969, suggesting that activation of 5-HT_{1B} receptors reduces primary reward as measured by ICSS (Harrison et al., 1999).

5-HT$_{1B}$ receptor-related ligands and self-administration

Drug self-administration is a behavioural test in which animals are trained to press a lever (or perform some other operant task) in order to obtain a drug injection. A drug is considered reinforcing if animals continue to lever press for administration of that drug. In addition, a drug may be considered to have abuse potential if animals administer the drug to the point of intoxication or tolerate aversive stimuli (e.g. electric shock) in order to obtain a drug injection. In addition, if food- or water- deprived animals choose drug administration over food or water intake, this may also be an indicator of the abuse potential of the drug (Gardner, 2000; Roberts and Goeders, 1989).

The selective 5-HT$_{1B}$ receptor agonist CP 94253, but not the mixed 5-HT receptor agonist RU 24969, was able to sustain self-administration in rats trained on a fixed ratio (i.e. a specific number of responses is required to receive administration of the drug) 5 schedule. However this effect was only evident at one dose tested and was only able to be maintained for up to 90 min (Parsons et al., 1998).

Cocaine self-administration

Pretreatment with the selective 5-HT$_{1B}$ receptor agonist CP 94253 or the mixed 5-HT receptor agonist RU 24969 induced a leftward shift in the dose-response curve in rats trained to administer cocaine on a fixed ratio 5 schedule. These agonists dose-dependently decreased self-administration of high doses of cocaine and enhanced the self-administration of a sub-threshold dose of cocaine. Similar effects were observed after intracerebroventricular administration of the 5-HT$_{1B}$ receptor agonist CP 93129 on cocaine self-administration (Parsons et al., 1998). In rats trained to administer cocaine on

a progressive ratio schedule (i.e. the number of responses required to receive administration of the drug increases after each drug delivery; e.g. the first drug delivery requires 1 lever presses, the second drug delivery requires 2 lever presses), Parsons et al. (1998) also found that CP 94253 and RU 24969 induced an increase in total cocaine intake through an increase in the cocaine breaking points (the point at which the animal will no longer respond for an injection), compared to the saline breaking point. The potentiating effect of CP 94253 on cocaine self-administration in a progressive ratio schedule was reduced by pretreatment with GR 127935. In addition, systemic administration of GR 127935 decreased intra-VTA cocaine self-administration in mice (David et al., 2004). In general, 5-HT_{1B} receptor activation appears to enhance the reinforcing effects of cocaine. This effect may be mediated in part by the VTA; however, further studies will be needed to confirm this notion.

Ethanol self-administration

Pretreatment with the selective 5-HT_{1B} receptor agonist CP 94253 reduced ethanol reinforced lever-pressing and ethanol preference in rats trained to orally administer ethanol on a fixed ratio 1 schedule (Maurel et al., 1999). Silvestre et al. (1998) also demonstrated a reduction in preference for a sweetened ethanol solution following pretreatment with the mixed 5-HT receptor agonist RU 24969. In addition, the mixed 5-HT receptor agonists RU 24969 and CGS 12066B decreased ethanol self-administration in animals trained on a fixed ratio 4 schedule of reinforcement (Tomkins and O'Neill, 2000). The effect of RU 24969 (except for the highest dose used) on ethanol self-administration was reversed by GR 127935 but not by the 5-HT_{1A} receptor antagonists

WAY 100135 or WAY 100635 (Tomkins and O'Neill, 2000). GR 127935 alone did not alter ethanol self-administration (Tomkins and O'Neill, 2000).

Amphetamine self-administration

Fletcher et al. (2002a) investigated the effects of the selective 5-HT_{1B} receptor agonist CP 93129 and the 5-$HT_{1B/D}$ receptor antagonist GR 127935 in rats trained to self-administer d-AMPH directly into the NAS on a progressive ratio schedule. CP 93129 reduced responding for d-AMPH, an effect attenuated by GR 127935. GR 127935 did not alter responding for d-AMPH when administered alone. The authors suggest that these data point to a somewhat selective role for NAS 5-HT_{1B} receptors in controlling the reinforcing properties of AMPH (Fletcher et al., 2002a). In addition, pretreatment with the mixed 5-HT receptor agonist RU 24969 reduced responding for d-AMPH in animals trained on a fixed ratio 1 schedule. This effect was blocked by the 5-$HT_{1B/D}$ receptor antagonist GR 127935 but not the 5-HT_{1A} receptor antagonist WAY 100635 (Fletcher and Korth, 1999).

In relation to the effects of 5-HT_{1B} receptor activation on the reinforcing properties of drugs of abuse, there appears to be mixed findings. In agreement with a decrease in reward observed following putative 5-HT_{1B} receptor activation seen in place conditioning and ICSS studies, agonists at this 5-HT receptor subtype decrease both AMPH and ethanol self-administration. However, the rewarding effects of cocaine seem to be enhanced by 5-HT_{1B} receptor activation. Differential results observed following 5-HT_{1B} receptor activation on self-administered compounds may be due to differences in training schedules or the mechanism of action of the self-administered compound.

5-HT_{2C} receptor-related ligands and place conditioning

Mosher et al. (2005) demonstrated that administration of the selective 5-HT_{2C} receptor agonist WAY 161503 or the mixed 5-HT receptor agonist TFMPP did not induce place conditioning when animals were tested in a drug-free state. In addition, the 5-HT_2 receptor agonist Ro 60-1075 was able to block the CPP induced by $^{\Delta 9}$-tetra-hydrocannabinol, the major active ingredient in marijuana (Ji et al., 2006). To date, no selective 5-HT_{2C} receptor antagonists have been studied in the place conditioning test; however, non-selective 5-HT_2 receptor antagonists with action at the 5-HT_{2C} receptor have been investigated using this behavioral test. The 5-HT_2 receptor antagonist ritanserin did not induce place conditioning alone, but was able to block the aversion (CPA) induced by phencyclidine and the preferences (CPP) induced by AMPH and diazepam respectively (Kitaichi et al., 1999; Noda and Nabeshima, 1998; Nomikos and Spyraki, 1988a). In addition, morphine-induced CPP was attenuated by ritanserin (Nomikos and Spyraki, 1988a).

The 5-HT_2 receptor antagonists mianserin and eltoprazine, but not ketanserin, were able to induce CPA (Rocha et al., 1993a). The acquisition of the CPAs induced by both mianserin and eltoprazine was blocked by administration of the mixed 5-HT receptor agonist mCPP; mCPP alone had no effect on place conditioning (Rocha et al., 1993a). Mianserin was also found to induce a CPA in rats lesioned with the neurotoxin 5,7- dihydroxytryptamine injected bilaterally into the lateral ventricles (Rocha et al., 1993b).

In contrast to Rocha et al. (1993a; 1993b), Risinger and Oakes (1996) demonstrated that mianserin was unable to induce place conditioning effects when

administered alone, but in combination with a sub-threshold dose of ethanol a CPP was observed. The differential effects observed regarding place conditioning induced by mianserin may be due to methodological or species differences between the two studies.

Although compounds which act as partial 5-HT_{2C} receptor agonists do not appear to induce place conditioning when administered alone, they may attenuate both the reinforcing and aversive properties of other compounds. Though these findings indicate opposing roles of 5-HT_{2C} receptor agonists on place conditioning, the discrepancies may be due to different interactions of the 5-HT receptor agonist with the compound of interest as the 5-HT_{2C} receptor-related drugs tested exhibit different receptor binding profiles. In general, blockade of the 5-HT_{2C} receptor appears to induce aversive reinforcing properties as measured by the induction of a CPA; however, as no selective 5-HT_{2C} receptor antagonists have been investigated using the place conditioning test, the role of other receptors such as the 5-HT_{2A} receptor in mediating this behaviour cannot be ruled out.

5-HT_{2C} receptor-related ligands and intracranial self-stimulation

Using the chronic stress-induced model of anhedonia (i.e. animals are exposed daily to unpredictable mild stressors such as food or water deprivation), Moreau et al. (1996) investigated effects of the 5-HT_2 receptor agonist Ro 60-0175 on rats responding for VTA ICSS. Ro 60-0175 prevented the development of the progressive increase in ICSS frequency induced by chronic mild stress; Ro 60-0175 was ineffective in non-stressed animals (Moreau et al., 1996). In addition, Ro 60-0175 treatment for three weeks during the stress period reversed the reward-decreasing effects of chronic mild

stress. These results may indicate potential antidepressant properties of the 5-HT_2 receptor agonist (Moreau et al., 1996).

The mixed 5-HT receptor agonist mCPP decreased response rates for medial forebrain bundle ICSS (Borisenko et al., 1996). In contrast, the 5-HT_2 receptor antagonist ketanserin (low dose) increased ICSS response rates at high current intensities. These results implicate 5-HT systems, specifically 5-HT_2 receptor subtypes, in lateral hypothalamic brain stimulation reward (Borisenko et al., 1996).

Moreau et al. (1994) investigated the effects of the mixed 5-HT_2 receptor antagonist mianserin on VTA ICSS using the chronic mild stress model of anhedonia. ICSS thresholds progressively increased over time in untreated animals; treatment with mianserin for 10 days during the stress period reversed threshold elevations to control levels. In addition, non-stressed animals did not experience any change in threshold measures; however, administration of mianserin for six days increased ICSS thresholds 20-30% (Moreau et al., 1994). These results may suggest that systemic blockade of 5-HT_2 receptors may reverse stress-induced anhedonia, but may also inhibit ICSS in non-stressed animals.

The mixed 5-HT receptor antagonist methysergide facilitated medial forebrain bundle response rates, but either decreased or had no effect on MRN response rates (Katz and Baldrighi, 1979). Methysergide treatment alone also did not affect response rates for lateral hypothalamic ICSS; however methysergide pretreatment did potentiate the reduction in response rates induced by the peptide somatostatin (Vecsei et al., 1983). The authors suggest that the serotonergic system may have a stimulatory, rather than

inhibitory role, on lateral hypothalamic ICSS, as blockade of 5-HT by methysergide further decreased responding induced by somatostatin (Vecsei et al., 1983).

In addition, methysergide did not affect response rates in rats implanted with lateral hypothalamic stimulating electrodes; methysergide pretreatment did potentiate response rates induced by AMPH. The authors attribute this effect to blockade of 5-HT receptors in a serotonergic system that inhibits ICSS (Silveira Filho and Graeff, 1977). Franklin and Robertson (1980) examined the effects of methysergide on AMPH responding for ICSS on various brain regions (medial PFC, lateral hypothalamus, dorsal tegmentum, or hippocampus). Methysergide administration alone did not alter animals' responding for ICSS of the medial PFC, lateral hypothalamus or dorsal tegmentum or affect response rates induced by AMPH in these areas. However, animals implanted with electrodes in the hippocampus were able to maintain low ICSS response rates. ICSS hippocampal response rates were unaffected by methysergide or l-AMPH treatment, but were enhanced by administration of d-AMPH. In addition, administration of methysergide and l-AMPH decreased hippocampal ICSS response rates; methysergide reversed, abolished, or facilitated the effect of d-AMPH. Co-administration of methysergide and d-AMPH was lethal in rats which demonstrated an increase in hippocampal ICSS response rates. The relevance of these findings to the role of 5-HT in reward is difficult to assess; the authors assert that 5-HT may play no direct role in ICSS of the medical PFC, dorsal tegmentum or lateral hypothalamus (Franklin and Robertson, 1980).

The mixed 5-HT antagonist metergoline induced a transient but significant decrease in response rates in animals implanted with electrodes in the MRN (Deakin,

1980). In addition, metergoline decreased response rates for habenula or MRN ICSS; decreases in later hypothalamic ICSS response rates were also observed, but they were not as pronounced as those seen in the habenula or MRN (Nakajima, 1984). Nakajima and McKenzie (1986) also demonstrated that administration of metergoline did not affect response rates for ICSS of the later hypothalamus, VTA , or DRN.

5-HT_{2C} receptor activation in the ICSS test by mCPP and Ro 60-0175 indicates that activation of this receptor may decrease or have no effect on reward. ICSS response rates were reported to be unaffected, increased or decreased following blockade of the 5-HT_{2C} receptor by non-selective 5-HT receptor drugs. Thus, the effect of 5-HT_{2C} receptor activation on reward as measured by ICSS is unclear from the current data. Differential results reported on ICSS may be due to differences in electrode implantation sites, receptor binding profile of the test compound or the measure of ICSS reward used (e.g. response rates vs. frequency thresholds). In order to clarify the effects of 5-HT_{2C} receptor stimulation/blockade in ICSS, future studies should employ specific ligands which act at this receptor site.

5-HT_{2C} receptor-related ligands and self-administration

Cocaine self-administration

Pretreatment with the selective 5-HT_{2C} receptor antagonist SB 242084 showed a dose-dependent increase in break-points in rats trained under a progressive ratio schedule. In addition, SB 242084 was shown to enhance cocaine reinstatement following a two-week extinction period (Fletcher et al., 2002b). In contrast, the 5-HT_{2C} receptor antagonist, SDZ SER-082 did not alter the maintenance of cocaine self-administration,

priming or cue-induced reinstatement (Filip, 2005) whereas the mixed 5-HT_2 receptor antagonist ritanserin did not affect cocaine-prime reinstatement of self-administration (Schenk, 2000) in rats trained on a fixed ratio 5 schedule of reinforcement.

Pretreatment with the mixed 5-HT receptor antagonists, methysergide or ketanserin did not alter response rates in animals trained to administer cocaine on a progressive ratio schedule (Lacosta and Roberts, 1993). Alternatively, ritanserin pretreatment decreased the intake of cocaine in rats trained to self-administer cocaine mixed into their water when they were presented with the choices of cocaine-water and water alone (Meert, 1991).

Ritanserin pretreatment also increased cocaine-mediated response rates in male squirrel monkeys trained under a second-order fixed interval schedule with a fixed ratio 20 component (Howell and Byrd, 1995). In addition, the mixed 5-HT receptor agonist mCPP or ketanserin decreased responding in squirrel monkeys trained to lever press for intramuscular injections of cocaine under a second-order schedule of reinforcement (Nader, 1990). In contrast, the mixed 5-HT receptor antagonist, metergoline significantly increased the response rates for cocaine self-administration.

Responding and reinstatement for cocaine self-administration was reduced by pretreatment with the 5-HT_2 receptor agonist Ro 60-0175 in rats trained under a fixed ratio 5TO1-min schedule of reinforcement (fixed ratio 5 schedule followed by a 1 min time-out period. During the time-out period the lights were turned off and when animals lever pressed no drugs were delivered) (Grottick et al., 2000). Ro 60-0175 also reduced the breakpoints for cocaine self-administration in rats trained under a progressive ratio schedule (Grottick et al., 2000). In a two-lever, water-reinforced fixed ratio 20 task,

where rats were trained to discriminate cocaine from saline, intra-NAS administration of the 5-HT_2 receptor antagonist RS 102221 reduced discriminability while the 5-HT_2 receptor agonists MK 212 or Ro 60-0175 enhanced discriminability of a sub-threshold doses of cocaine (Filip and Cunningham, 2002). In addition, intra-VTA infusion of Ro 60-0175 decreased cocaine self-administration in rats trained on both a fixed ratio 5 or progressive ratio schedule; this effect of Ro 60-0175 on cocaine self-administration in the progressive ratio schedule was blocked by systemic administration of selective 5-HT_{2C} receptor antagonist SB 242084 (Fletcher et al., 2004). The 5-HT_{2C} receptor antagonist SDZ SER-082 induced a leftward shift in the dose-response curve in rats trained to discriminate cocaine from saline in a two-lever water-reinforced fixed ratio 20 task (Filip et al., 2006).

Ethanol self-administration

In low ethanol drinking rats, pretreatment with the selective 5-HT_{2C} receptor antagonist SB 242084 increased responding for ethanol on a fixed ratio 4 schedule of reinforcement (Tomkins et al., 2002). In contrast, the 5-HT_2 receptor antagonist ritanserin decreased ethanol self-administration and ethanol preference in a two-choice (water vs. ethanol) test in rats (Meert, 1991). However, ritanserin administration reduced ethanol intake in rats demonstrating a high or medium preference for a 3% ethanol solution when presented with a two-bottle choice (water vs. ethanol) test (Meert, 1993). Furthermore, Silvestre et al. (1998) observed a reduction in fluid intake without a change in ethanol preference following treatment with ritanserin.

Administration of the mixed 5-HT receptor agonist TFMPP also decreased responding for ethanol self-administration (Wilson et al., 1998; Wilson et al., 2000). Similarly, the 5-HT_2 receptor agonist Ro 60-0175 dose-dependently decreased responding, time spent responding and total intake of ethanol in rats on a fixed ratio 4 schedule; the effect of Ro 60-0175 on ethanol self-administration was reversed by SB 242084 (Tomkins et al., 2002).

Amphetamine self-administration

Pretreatment with ritanserin was unable to alter response rates for rats trained to self-administer d-AMPH on a progressive ratio schedule (Fletcher, 1998).

Results from self-administration studies of both cocaine and ethanol have demonstrated that blockade of the 5-HT_{2C} receptor may potentiate the reinforcing effects of these compounds but may not affect those of AMPH. At least one study however, suggested that blockade of the 5-HT_{2C} receptor has no effect on cocaine self-administration, priming or cue-induced reinstatement. The differential conclusions reached by each study may be due to inter-laboratory differences or to the sensitivity of the various compounds used to block the 5-HT_{2C} receptor. In contrast, compounds which act as partial 5-HT_{2C} receptor agonists may decrease the reinforcing properties of both cocaine and ethanol. These results are consistent with reward decreasing effects observed following 5-HT_{2C} receptor stimulation in other behavioural tests such as ICSS. Due to the previous lack of selective 5-HT_{2C} receptor agonists, the exact role this receptor plays in reward and reinforcement has not been thoroughly investigated; this should be addressed in future studies.

Nicotine-induced hyperactivity

Repeated administration of nicotine induces sensitization of mesocorticolimbic DA neurons and locomotor hyperactivity. Systemic nicotine administration increases both the burst firing of DA neurons in the VTA (Mereu et al., 1987; Nisell et al., 1996) as well as VTA (Rahman et al., 2003), NAS (Benwell and Balfour, 1992; Benwell et al., 1995; Damsma et al., 1989; Imperato et al., 1986; Nisell et al., 1994b; Nisell et al., 1996; Olausson et al., 2001) and medial PFC (Clarke et al., 1988; Nisell et al., 1996) extracellular DA concentrations. In addition, NAS DA release is increased following intra-VTA or intra-NAS nicotine administration (Nisell et al., 1994a). The effects on NAS DA release and VTA DA-neuronal burst firing were respectively reversed by intra-VTA or systemic administration of the centrally acting nicotinic acetylcholine (nAch) receptor antagonist mecamylamine (Mereu et al., 1987; Nisell et al., 1994b).

Repeated systemic nicotine administration induces locomotor hyperactivity in rats (Clarke and Kumar, 1983b; Morrison and Stephenson, 1972; Stolerman et al., 1973). In addition, intra-VTA administration of nicotine or the nAch receptor agonist cytisine may also induce locomotor stimulant effects (Museo and Wise, 1990, 1994, 1995; Reavill and Stolerman, 1990). Nicotine-induced hyperactivity was blocked by systemic administration of the nAch receptor antagonist mecamylamine but not the peripherally acting nAch receptor antagonists chlorisondamine or hexamethonium (Benwell et al., 1995; Clarke and Kumar, 1983a, b). The action of nicotine on central nAch receptors and on mesocorticolimbic DA neurons appears to be responsible for the induction of nicotine-induced locomotor hyperactivity. In this thesis, nicotine-induced hyperactivity was used as an index of mesocorticolimbic DA function. As 5-HT_{1B} or 5-HT_{2C} receptor

stimulation is known to alter mesocorticolimbic extracellular DA levels, the behavioural effects on nicotine-induced hyperactivity following activation of these 5-HT receptor subtypes were examined in this thesis.

General Methodology

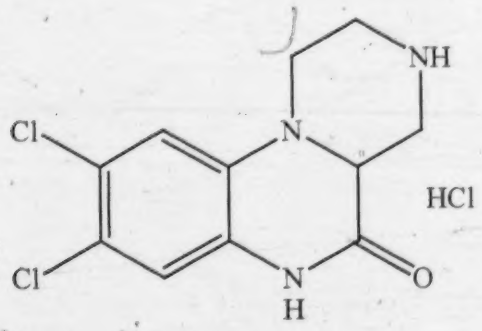
In all studies described in this thesis, drugs were administered either systemically (subcutaneously - s.c. or intraperitoneally - i.p.) or centrally into the VTA by microinjection. In order to microinject compounds into the VTA, rats were stereotaxically implanted with a 22-gauge stainless steel guide cannula which was fixed to the skull using stainless steel screws (3.6 mm long and 0.8 mm in diameter) and dental acrylic. The microinjection procedure began at least one week post-surgery at which time behavioural responses to the compound of interest were assessed.

Drugs

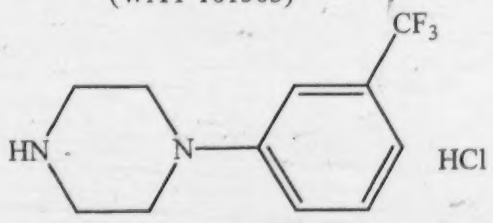
The following drugs were purchased from the Sigma Chemical Company (St. Louis, MO, USA): (-)- nicotine hydrogen tartrate, the nAch receptor agonist (-) -cytisine, the mixed 5-HT receptor agonist N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride (TFMPP), the selective 5-HT_{2C} receptor antagonist 6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride (SB 242084) and the selective 5-HT_{1A} receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl-cyclohexanecarboxamine maleate (WAY 100635). The selective 5-$HT_{1B/D}$ receptor antagonist N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride (GR

127935), the selective 5-HT_{2C} receptor agonist 8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride (WAY 161503), the mixed 5-HT receptor agonist 5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole hemisuccinate (RU 24969) and the selective 5-HT_{1B} receptor agonist 5-Propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine hydrochloride (CP 94253) were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+) –amphetamine sulphate (AMPH) was purchased from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). Chemical structures of the drugs used in this thesis are shown in Figure 1.7.

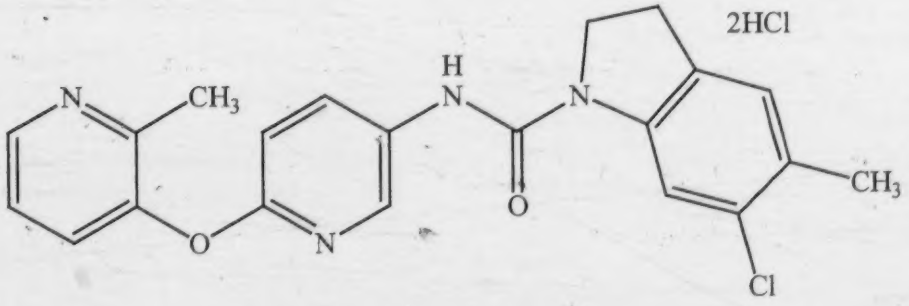
Figure 1.7. Chemical structures of WAY 161503, TFMPP, SB 242084, CP 94253, RU 24969, GR 127935, WAY 100635, (+)-AMPH, (-)-nicotine and (-)-cytisine.



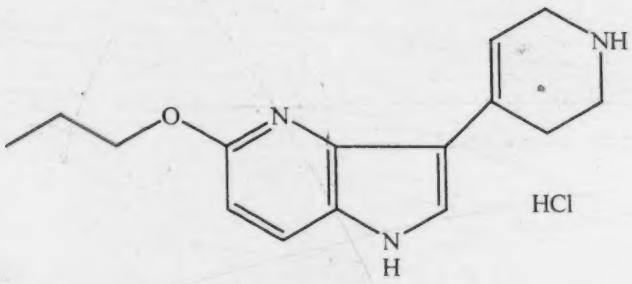
8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride
(WAY 161503)



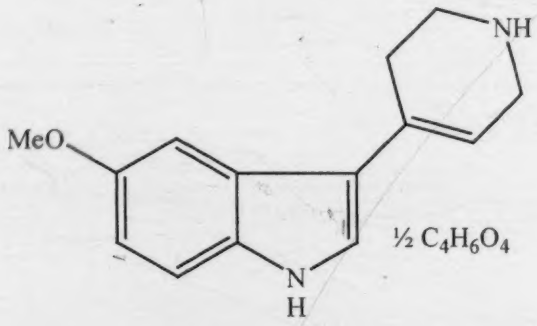
N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride (TFMPP)



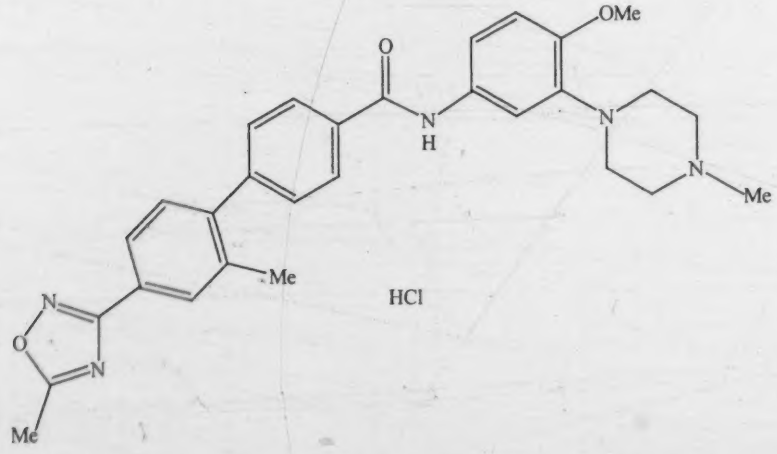
6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline
dihydrochloride
(SB 242084)



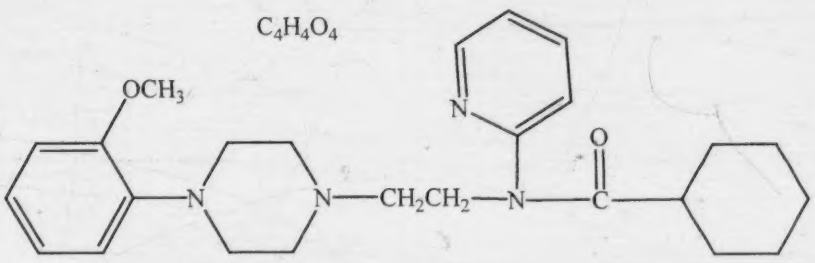
5-Propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine hydrochloride
(CP 94253)



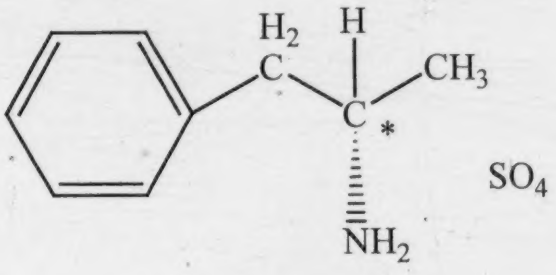
5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole hemisuccinate
(RU 24969)



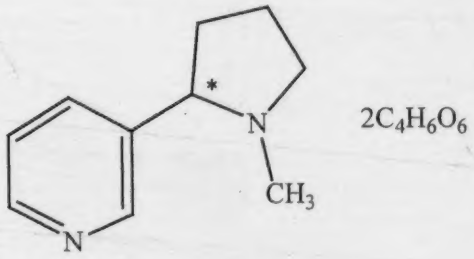
N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride (GR 127935)



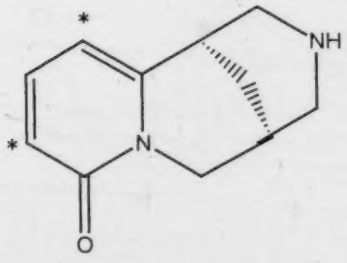
N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl-cyclohexanecarboxamine maleate (WAY 100635)



(+) – AMPH sulphate



(-) – Nicotine bitartrate



(-) – Cytisine

Locomotor activity

Both the acute and chronic effects of drugs on locomotor activity were studied in this thesis. Locomotor activity is a widely used behavioural test to measure the stimulant or depressant effects of drugs. Locomotor activity is measured in our laboratory using Plexiglas boxes (43 cm L x 43 cm W x 30 cm H), each lined with a 12x12 photobeam grid located 2.5 cm above the floor to measure horizontal and consecutive beam breaks. In addition, there are an additional 12 photobeams located 12 cm above the floor to measure vertical (rearing) activity. Photobeams originate from a light source on one side of the box and travel to a light-activated switch on the opposite side. When a photobeam is broken, this is measured and recorded on a computer to account for horizontal, consecutive and vertical (rearing) activity. Local activity counts are tabulated every 5 min for the duration of the testing session. During each locomotor activity experiment, the following measurements are recorded:

Horizontal Activity - total number of lower photobeam breaks

Consecutive Activity - repetitive breaking of one photobeam

Vertical Activity - total number of upper photobeam breaks

Place conditioning

Place conditioning is a behavioural test used to measure the conditioned reinforcing effects of a stimulus (e.g. drugs, food, access to a sexual partner). Briefly, place conditioning occurs when two previously neutral but distinct environments are paired with two distinct unconditioned stimuli (i.e. drug and vehicle treatment). Following conditioning, when the animals are given free access to both environments, the

time spent in each environment is used as an index of the rewarding or aversive properties of the unconditioned stimulus (Swerdlow et al., 1989; Tzschentke, 1998).

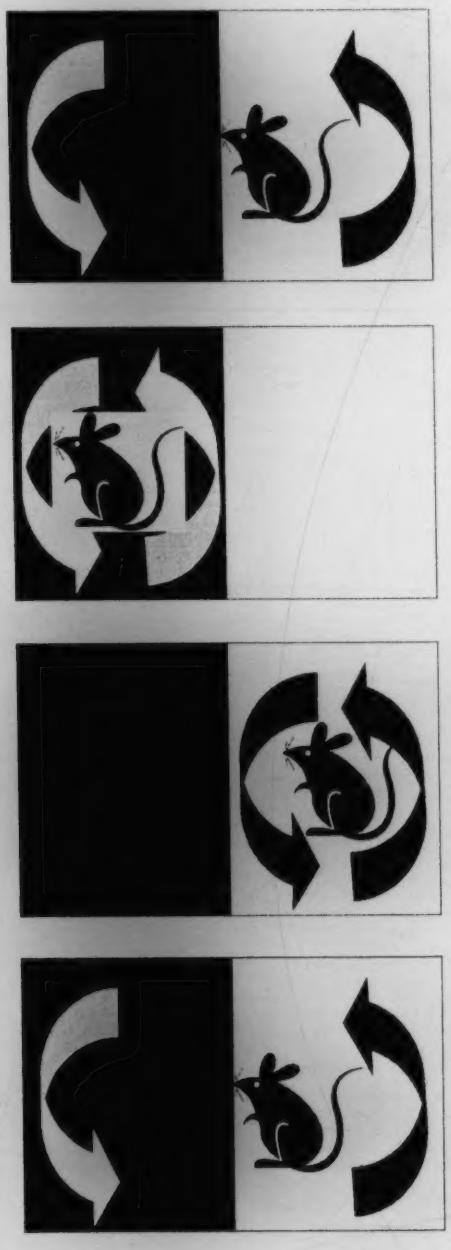
In this thesis, animals were exposed to two distinct neutral environments which varied only by floor texture (1-cm square grate wire compared with 14 horizontal bars positioned 1.25 cm apart). Time spent in each compartment was recorded and used as a measure of baseline compartment preference. Animals were assigned to drug treatment groups; one environment was paired with the drug treatment and the other environment with vehicle treatment. Following drug-compartment pairings, animals were given free access to both environments in either a drug-free or drugged state. Time spent in each compartment was recorded and the induction of a CPP or CPA was determined by comparing the animals' baseline preference to the time spent in the drug-paired side following conditioning. A simplified version of the place conditioning procedure is outlined in Figure 1.8.

State-dependent place conditioning

State dependency refers to a behaviour or response which was acquired while in a given state (e.g. drugged-state) and can only be expressed if the animal is tested in the same state as it was conditioned (Tzschentke, 1998). Previous studies have shown that compounds such as diazepam, cocaine, morphine, naxolone and lithium are able to induce place conditioning when animals are tested in a drugged state (Herzig and Schmidt, 2004; Mucha and Iversen, 1984; Nomikos and Spyraki, 1988b; Oberling et al., 1993; Olmstead and Franklin, 1997; Sakoori and Murphy, 2005; Spyraki et al., 1985). In this thesis, to test for state-dependency, the same procedure was followed as that used for

testing animals in a drug-free state with the exception that on the first day of retention testing, animals received an injection of the drug to which they were conditioned.

Figure 1.8. Schematic drawing of a simply place conditioning procedure. Adapted from Swerdlow et al. (1989).



Pre-Conditioning
Animals have access to entire apparatus for three days (15 min/day) in a drug-free state

Conditioning
Animals are injected with either drug or vehicle on alternate days for six days and confined to one side of the apparatus for 30 min/day.

Post-Conditioning
Animals again have free access to the entire apparatus for three days (15 min/day).

Conditioned taste aversion

Conditioned taste aversive (CTA) is a behavioural paradigm that is known to be very sensitive for measuring the reinforcing stimulus properties of drugs (Goudie, 1986). The CTA procedure is generally thought be an associative learning paradigm in which animals associate the consumption of a novel food with a change in the internal environment. In this thesis, animals were exposed to a 0.1% saccharin solution following which; animals received an injection of their assigned drug treatment. It is interesting to note that drugs of abuse such as nicotine, AMPH, morphine and cocaine that are rewarding in other behavioural tests all induce CTA learning (Etscorn et al., 1987; Goudie et al., 1978; Greenshaw and Dourish, 1984; Kumar et al., 1983; LeBlanc and Cappell, 1975; Miller and Miller, 1983; Pilcher and Stolerman, 1976; Van der Kooy and Phillips, 1977). Although the reason why drugs of abuse induce CTA remains unknown, it has been proposed that, from an evolutionary perspective, CTA learning may be related to the avoidance of noxious substances in relation to ingestion (Goudie, 1986; Mediavilla et al., 2005).

For CTA experiments, animals were placed on restricted water access (access to water for 30 min/day) for eight days in order to determine baseline water consumption. Following the determination of baseline water consumption, animals were randomly assigned to drug-treatment groups. For the next three days, animals had access to two bottles of 0.1% saccharin solution for 30 min/day. Immediately following the end of the saccharin drinking sessions, animals were injected with their assigned drug-treatment. Following the saccharin conditioning days, extinction testing took place over a three day period. During this time, subjects were given a two-bottle choice test with access to one

bottle of saccharin solution and one bottle of water for 30 min/day. The induction of a CTA was determined by comparing the percent of saccharin consumed in the drug-treated animals compared to those treated with saline.

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Chapter 2. Differential effects of 5-HT_{2C} receptor ligands on place conditioning and locomotor activity in rats

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Introduction

Central 5-HT-containing neurons originate from the midbrain raphé nuclei and project to the VTA, the substantia nigra and the NAS (Azmitia and Segal, 1978; Herve et al., 1987; Moukhles et al., 1997). The actions of synaptically released 5-HT are mediated by actions among at least fourteen distinct structural and pharmacological subtypes of 5-HT receptors (Barnes and Sharp, 1999). Activation of these receptor subtypes are involved in a variety of behavioural responses including: sleeping, feeding, memory, pain, sexual activity, locomotion, and positive and negative reinforcement (Barnes and Sharp, 1999; Jacobs and Fornal, 1999; Jouvet, 1999). In particular, activation of the 5-HT_{2C} receptor has been associated with decreased locomotor activity, hypophagia, anxiety, penile erections and hyperthermia (Barnes and Sharp, 1999; Koek et al., 1992).

Currently, it is hypothesized that the mesocorticolimbic DA system plays a role in reward mediated behaviour (Tzschentke, 1998; Wise and Rompre, 1989). Within the mesocorticolimbic system, DA has significant interactions with 5-HT; neurons containing 5-HT may play an inhibitory role with respect to DA (Di Giovanni et al., 2000; Di Matteo et al., 2000a; Tzschentke, 1998). 5-HT receptor subtypes may play a functional role in the regulation of neural processes underlying motivation and reward. Systemic

administration of the 5-HT_{1A} receptor agonist 8-OHDPAT alters lateral hypothalamic reward thresholds, with lower doses decreasing and higher doses increasing thresholds, respectively in two studies (Harrison and Markou, 2001; Montgomery et al., 1991). Ahn et al. (2005) have reported a monotonic increase in reward for self-stimulation at ventral tegmental electrode sites over a wide systemic range of doses of 8-OHDPAT. Systemically administered 8-OHDPAT also decreased self-administration of cocaine and ethanol (Burmeister et al., 2004; Parsons et al., 1998; Peltier and Schenk, 1993; Roberts et al., 1998). Nevertheless, when this drug was administered directly into the DRN or MRN, respectively, a decrease in ICSS reward thresholds was observed (Ahn et al., 2005; Fletcher et al., 1995; Harrison and Markou, 2001). In agreement with these findings, stimulation of the 5-HT_{1A} receptor may result in a CPP (Fletcher et al., 1993; Neisewander et al., 1990; Papp and Willner, 1991). These results have generally been interpreted in terms of an action of 8-OH-DPAT at somatodendritic 5-HT_{1A} receptors on 5-HT neurons (reward increasing effects) or post-synaptic 5-HT_{1A} receptors (reward decreasing effects, see Ahn et al. (2005)). 5-HT_{1B} receptor activation may also be relevant to motivation and reward. Activation of the 5-HT_{1B} receptor may induce a CPP when administered with a sub-threshold dose of cocaine (Cervo et al., 2002) and has been observed to facilitate sub-threshold levels of cocaine self-administration (Parsons et al., 1998). By contrast, activation of that receptor may reduce both responding for AMPH self-administration (Fletcher et al., 2002a) and ICSS reward thresholds (Harrison et al., 1999) as well as induce a CPA (Cervo et al., 2002).

With regard to the 5-HT_2 family of receptors, few studies have examined the effects of specific ligands in behavioural tests used to measure reward. It has been shown

that 5-HT_{2A} receptor blockade does not alter the effects of AMPH on ICSS reward thresholds (Moser et al., 1996), but attenuates the reinstatement of cocaine self-administration (Fletcher et al., 2002b). Furthermore, unpublished data from our laboratory has shown that 5-HT_{2C} receptor stimulation decreases ICSS reward thresholds whereas the 5-HT_{2C} receptor antagonist SB 242084 facilitates ICSS reward (Clements RLH, personal communication). In agreement with these results, 5-HT_{2C} receptor blockade has been demonstrated to increase cocaine breaking points in self-administration (Fletcher et al., 2002b) and increase responding for low ethanol drinking rats (Tomkins et al., 2002).

The 5-HT_{2C} receptor is a G-protein coupled receptor which activates phospholipase C and increases phosphotidyl inositol hydolysis (Barnes and Sharp, 1999; Boess and Martin, 1994). Available data indicate that 5-HT_{2C} receptors are located on GABA-containing neurons but not on DA neurons (Di Giovanni et al., 2001; Eberle-Wang et al., 1997; Serrats et al., 2005). In vivo, 5-HT_{2C} receptor activation reduced activity in the mesocorticolimbic DA system (Di Giovanni et al., 2000; Di Matteo et al., 1999, 2000a; Di Matteo et al., 2000b; Di Matteo et al., 2001; Di Matteo et al., 2002; Millan et al., 1998; Pozzi et al., 2002). As 5-HT_{2C} receptors are not expressed on DA neurons, the decrease in DA release observed after activation of the 5-HT_{2C} receptor may be mediated by activation of GABA interneurons in the VTA (Di Giovanni et al., 2001; Di Matteo et al., 2001; Di Matteo et al., 2002). Few studies have examined the positive or negative reinforcing effects of 5-HT_{2C} receptor ligands in place conditioning. Rocha et al. (1993) found that the 5-$HT_{1B/2C}$ receptor agonist mCPP did not induce place conditioning on its own, but was able to block the CPA induced by mianserin (mixed 5-

HT_2 receptor antagonist) and eltoprazine (mixed 5-HT_{1B} receptor agonist/5-HT_{2C} receptor antagonist). Selective 5-HT_{2C} receptor ligands have recently been developed, allowing for the behavioural characterization of these receptors in place conditioning.

The effects of the 5-HT_{2C} receptor WAY 161503 on locomotor activity *per se* have not been reported, but WAY 161503 decreased immobility and increased swimming in the forced swim test (Cryan and Lucki, 2000). These effects were attenuated by pretreatment with the 5-HT_{2C} receptor antagonist SB 206533 and the non-specific 5-HT_2 receptor antagonist mianserin. Administration of 5-HT_{2C} receptor antagonists such as SB 242084 does not result in significant changes in locomotor activity (Kennett et al., 1997; Martin et al., 2002).

The mixed 5-$HT_{1B/2C}$ receptor agonist mCPP reduces locomotor activity, an effect that is blocked by pretreatment with SB 242084 (Gleason et al., 2001; Kennett et al., 1997; Martin et al., 2002). In addition, a dose-dependent reduction in locomotor activity has been reported following administration of the mixed 5-HT_2 receptor agonist Ro 60-0175, acting at the 5-$HT_{2A/B/C}$ receptor subtypes, an effect that was reversed by pretreatment with SB 242084 (Higgins et al., 2001; Kennett et al., 2000). SB 242084 also potentiated the increase in locomotor activity induced by the indirect 5-HT releaser/reuptake inhibitor (+) fenfluramine (Higgins et al., 2001).

Lucki et al. (1989) reported a dose-dependent reduction in locomotor activity following administration of the mixed 5-HT (5-$HT_{1A/B/2C}$) receptor agonist TFMPP (0.31-5.0 mg/kg) that was not blocked by pindolol and propranolol: compounds which are potent β-adrenoceptor blockers but also act as potent antagonists of the 5-$HT_{1A/B}$ and 5-HT_{1B} receptors, respectively. These effects were decreased by the non-selective 5-HT

receptor antagonists metergoline, methysergide and mianserin. These authors hypothesized that these locomotor inhibitory effects of TFMPP may be due to 5-HT_{2C} receptor activation (Lucki et al., 1989), in accordance with the above studies that indicate a possible role for 5-HT_{2C} receptors in the regulation of exploratory motor behaviour.

To explore the potential role of 5-HT_{2C} receptors in place conditioning, WAY 161503 and TFMPP were assessed using this behavioural test. It was hypothesized that these compounds may induce a CPA, as activation of 5-HT_{2C} receptors by Ro 60-0175 and MK 212 decreases mesocorticolimbic DA release from areas such as the frontal cortex, NAS and VTA (Di Giovanni et al., 2000; Di Matteo et al., 2000a; Di Matteo et al., 2001; Millan et al., 1998). The present study also attempted to clarify the role of 5-HT_{2C} receptors in the regulation of spontaneous locomotor activity. It was hypothesized that the systemic effects of the selective 5-HT_{2C} receptor agonist WAY 161503 and of the less selective 5-HT receptor agonist TFMPP on spontaneous locomotor activity would be attenuated or blocked by administration of the 5-HT_{2C} receptor antagonist SB 242084 but not by administration of a 5-HT_{1A} receptor antagonist WAY 100635 or a 5-$HT_{1B/D}$ receptor antagonist GR 127935. Based on previous studies, it was hypothesized that a) SB 242084 alone would not alter locomotor activity and b) TFMPP and WAY 161503 would decrease locomotor activity and that these effects may be attenuated by pretreatment with SB 242084.

Materials and Methods

Animals

Male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-250 g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights from 0700 h -1900 h) with food and water freely available. The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Place Conditioning

Apparatus: The place conditioning apparatus (I. Halvorson System Design, Phoenix, AZ, USA) consisted of a rectangular Plexiglas box divided into two compartments (30 cm L x 30 cm W x 25 cm H). The compartments differed in floor texture: 14 horizontal bars positioned 1.25 cm apart compared with 1-cm square grate wire flooring. The compartments were separated by a white plastic divider, which contained a tunnel (7.5 cm long) allowing access to both compartments that could be obstructed with removable doors during conditioning.

Procedure: The procedure consisted of three phases. *Phase1* (Pre-Conditioning): Animals (n=8) were habituated to the place conditioning apparatus for three consecutive days, during which animals had free access to both compartments for 15 min. On the third day of pre-conditioning, the amount of time spent in each compartment was recorded. Independent experiments were conducted according to an unbiased and a

biased design. For the unbiased design, animals were randomly assigned to each drug group and adjusted if necessary to equalize baseline compartment preference between and within each group.

In the biased design, because induction of a CPA was expected following administration of the 5-HT_{2C} receptor agonist, animals were randomly assigned to drug groups such that they were each conditioned to the most preferred compartment, as determined on pre-conditioning day three. In the biased design the (+)-AMPH sulphate positive control group was conditioned to the least preferred side, as determined on pre-conditioning day three. This was arranged because induction of a CPP was expected in that group. *Phase2* (Conditioning): On alternating days, animals received drug and vehicle treatment and were confined to the drug-paired or vehicle-paired compartment for 30 min. Animals were conditioned for six consecutive days (Bilsky and Reid, 1991; Kankaanpaa et al., 2002; Shippenberg, 1991). *Phase3* (Post-Conditioning): During retention testing, animals were placed in the apparatus; in a drug-free state and allowed free access to both compartments for 15 min. The amount of time spent in each compartment was recorded to the nearest second.

All testing took place under red light during the light phase of the light/dark cycle. The place conditioning apparatus was cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name ® Glass Cleaner with ammonia).

Spontaneous Locomotor Activity

Apparatus: Spontaneous locomotor activity was measured using computer–monitored photobeam boxes (I. Halvorson System Design, Phoenix, AZ, USA). The

locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity as well as consecutive beam breaks. Vertical activity was measured using 12 additional photobeams located 12 cm above the floor.

Procedure: Animals (n=8) were habituated to the locomotor activity boxes for two consecutive days (60 min/day) to establish baseline exploratory activity. Animals then received four randomized counterbalanced injections with three drug-free days between injections. Locomotor activity was monitored for 60 min during the WAY 161503 and SB 242084 dose response experiments to explore the time course of drug effects. Locomotor activity was monitored for 30 min during TFMPP experiments, as TFMPP may alter activity during the first 30 min of testing (Waddock, 1997). All testing took place under red light during the light phase of the light/dark cycle. The photobeam boxes were cleaned between animals with ammonia-based window cleaner (No Name ® Glass Cleaner with ammonia) diluted with water (1:6).

Drugs

The 5-$HT_{1A/B/2C}$ receptor agonist TFMPP · HCl [N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride], the 5-HT_{2C} receptor antagonist SB 242084 · 2HCl [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride] and the 5-HT_{1A} receptor antagonist WAY 100635 maleate [N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl-cyclohexanecarboxamine maleate] were purchased from Sigma Chemical Company (St. Louis, MO, USA). The 5-$HT_{1B/D}$ receptor antagonist GR 127935 · HCl [N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-

methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride], and the 5-HT_{2C} receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+)- AMPH sulphate was purchased from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). AMPH, TFMPP, and WAY 100635 were dissolved in 0.9% saline. SB 242084, GR 127935 and WAY 161503 were dissolved in doubled distilled water. SB 242084 was injected i.p. and all other drugs were injected s.c. in a volume of 1.0 ml/kg. Drug doses are expressed as free-base.

Drug Treatment

Experiment 1: The effects of systemic TFMPP or WAY 161503 on place conditioning

The effects of TFMPP (3.0 mg/kg, s.c., 10 min prior) on place conditioning were examined. Animals received three injections of TFMPP and vehicle on alternating days. The effects of TFMPP were tested in both an unbiased and biased place conditioning design.

The effects of WAY 161503 (1.0 or 3.0 mg/kg, s.c., 10 min prior) on place conditioning in an unbiased design were examined. Animals received three injections of WAY 161503 and vehicle on alternating days. In addition, the effects of WAY 161503 (3.0 mg/kg, s.c., 10 min prior) on place conditioning in a biased design (n=8) was also assessed. Animals received three injections of WAY 161503 and vehicle on alternating days. The doses of TFMPP and WAY 161503 were chosen based on previous work in this laboratory and Cryan and Lucki (2000).

For all treatments, a positive control group was included. These animals received (+) - AMPH (1.0 mg/kg, s.c., 10 min prior) and vehicle on alternating days.

Experiment 2: Effect of systemic SB 242084 on locomotor activity

Eight animals received a randomized, counterbalanced series of four injections of either vehicle or SB 242084 (0.3, 1.0 or 3.0 mg/kg, i.p., 30 min prior to testing) and activity was measured for 60 min. Three days of vehicle treatment test days intervened between drug injection days.

Experiment 3: Effect of systemic WAY 161503 and SB 242084 on locomotor activity

Eight animals received a randomized, counterbalanced series of four injections of either vehicle or WAY 161503 (0.3, 1.0 or 3.0 mg/kg, s.c., 10 min prior to testing) and activity was measured for 60 min. Following completion of that experiment, each animal received an injection of SB 242084 (1.0 mg/kg, i.p., 30 min prior) followed by an injection of WAY 161503 (3.0 mg/kg, s.c., 10 min prior) on a single test day. This dose of SB 242084 was chosen based on prior studies (Martin et al., 2002). Three days of vehicle treatment test days intervened between drug injection days.

Experiment 4: Effects of 5-HT receptor antagonists on TFMPP induced changes in locomotor activity

Separate groups of animals (n=8), received four randomized counterbalanced injections as follows: TFMPP (3.0 mg/kg, s.c., 10 min prior) and SB 242084 (1.0 mg/kg, i.p., 30 min prior) alone, in combination and with control; TFMPP (3.0 mg/kg, s.c., 10 min prior) and WAY100635 (0.1 mg/kg, s.c., 15 min prior) alone, in combination and with control; TFMPP (3.0 mg/kg, s.c., 10 min prior) and GR 127935 (3.0 mg/kg, s.c., 30 min prior) alone, in combination and with control. The dose of TFMPP was chosen based

on previous work in this laboratory (Waddock, 1997). Three days of vehicle treatment test days intervened between drug injection days.

Statistical Analysis

Experimental effects in the place conditioning experiments were determined by paired sample t-tests to compare time spent in the drug-paired side on pre-conditioning day 3 and post-conditioning day 1 ($P \leq 0.05$, n=8 per group). Experimental effects on spontaneous locomotor activity were determined using a one-way repeated measures analysis of variance (ANOVA). WAY 161503 + SB 242084 and SB 242084 dose–response data were analyzed using two-way repeated measures ANOVA (drug x time). Drug interaction data for experiments involving TFMPP were analyzed using three-way repeated measures ANOVA [TFMPP x antagonist x time]. Where appropriate, analysis of time course data using one-way repeated measures ANOVA across all drug groups at each 5 min interval was conducted. A significant F ratio ($P \leq 0.05$) on a 5 min interval was followed by comparison of each drug condition to vehicle using Newman-Keul's post hoc tests (α=0.05). As the results of the analyses of consecutive and vertical activity paralleled those for horizontal locomotor activity counts, only the latter results are reported. All statistical analyses were completed using SPSS statistical software (SPSS 11.5, SPSS Inc., Chicago, U.S.A.).

Results

Experiment 1: The effect of systemic TFMPP or WAY 161503 on place conditioning

TFMPP did not induce place conditioning in either an unbiased [$t(7) = 1.51$, $P > 0.05$] or biased design [$t(7) = 1.41$, $P > 0.05$] (Figure 2.1A and B); under these

conditions (+)- AMPH induced a significant CPP [t (7) = 4.987, P< 0.05, unbiased; t (7) = 4.845, P< 0.05, biased]. The 5-HT_{2C} receptor agonist WAY 161503 did not induce place conditioning in an unbiased [1.0 mg/kg t (7) = 0.46, P >0.05; 3.0 mg/kg t (7) = 0.12, P >0.05] (Figure 2.2A) or biased [3.0 mg/kg t (7) = 0.25, P >0.05] design (Figure 2.2B). Under these conditions (+)-AMPH induced a significant CPP [t (7) = 3.281, P < 0.05, unbiased; t (7) = 4.878, P < 0.05, biased].

Figure 2.1. Effects of TFMPP (3.0 mg/kg) and (+) - AMPH (1.0 mg/kg) with unbiased (A) and biased (B) place conditioning procedures. Data shown are means ± S.E.M.

* Significant $P<0.05$.

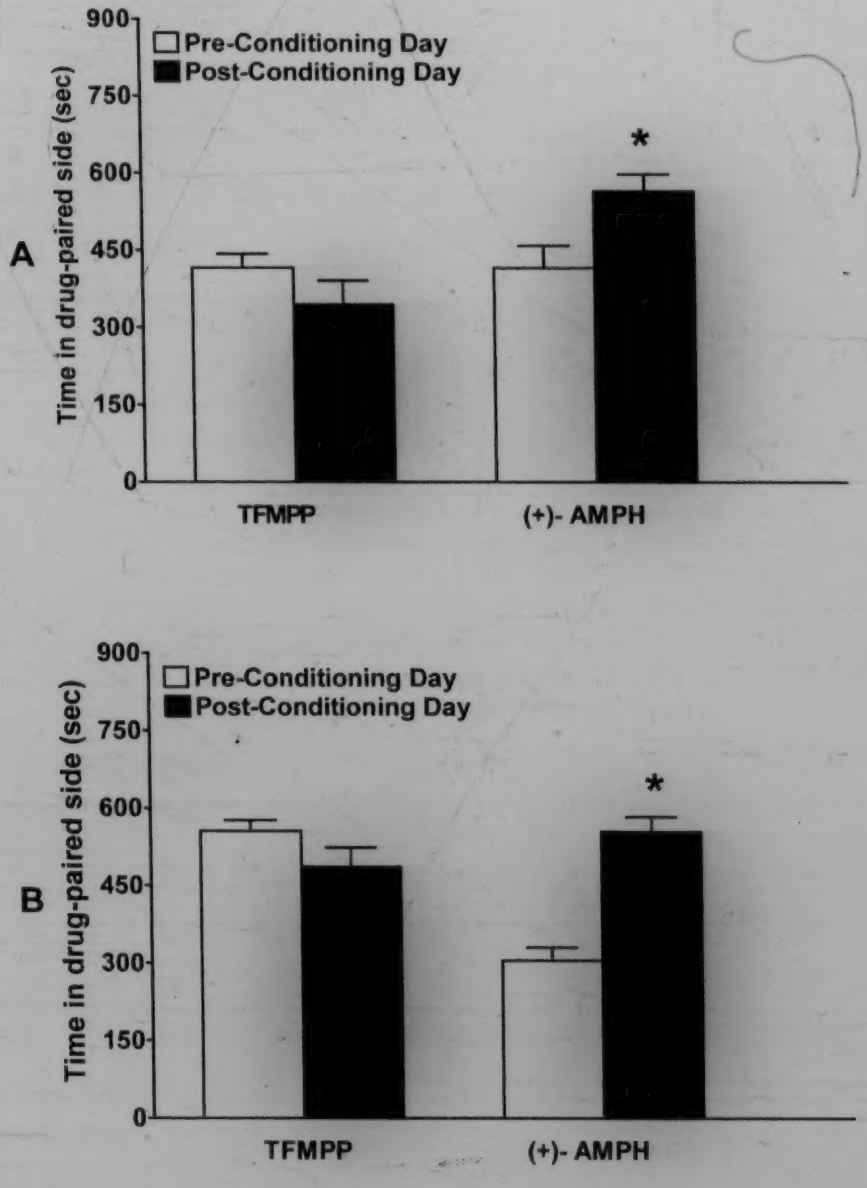
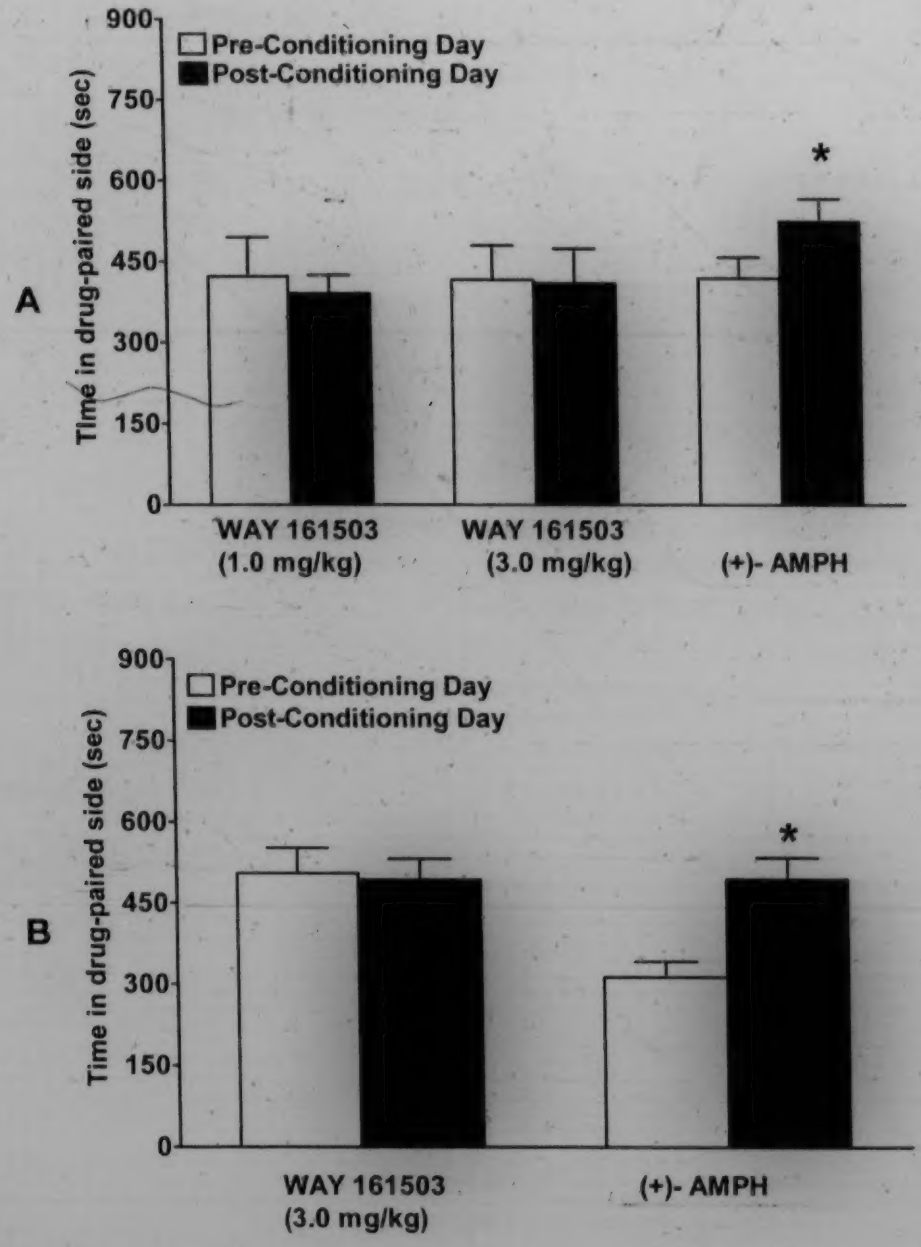


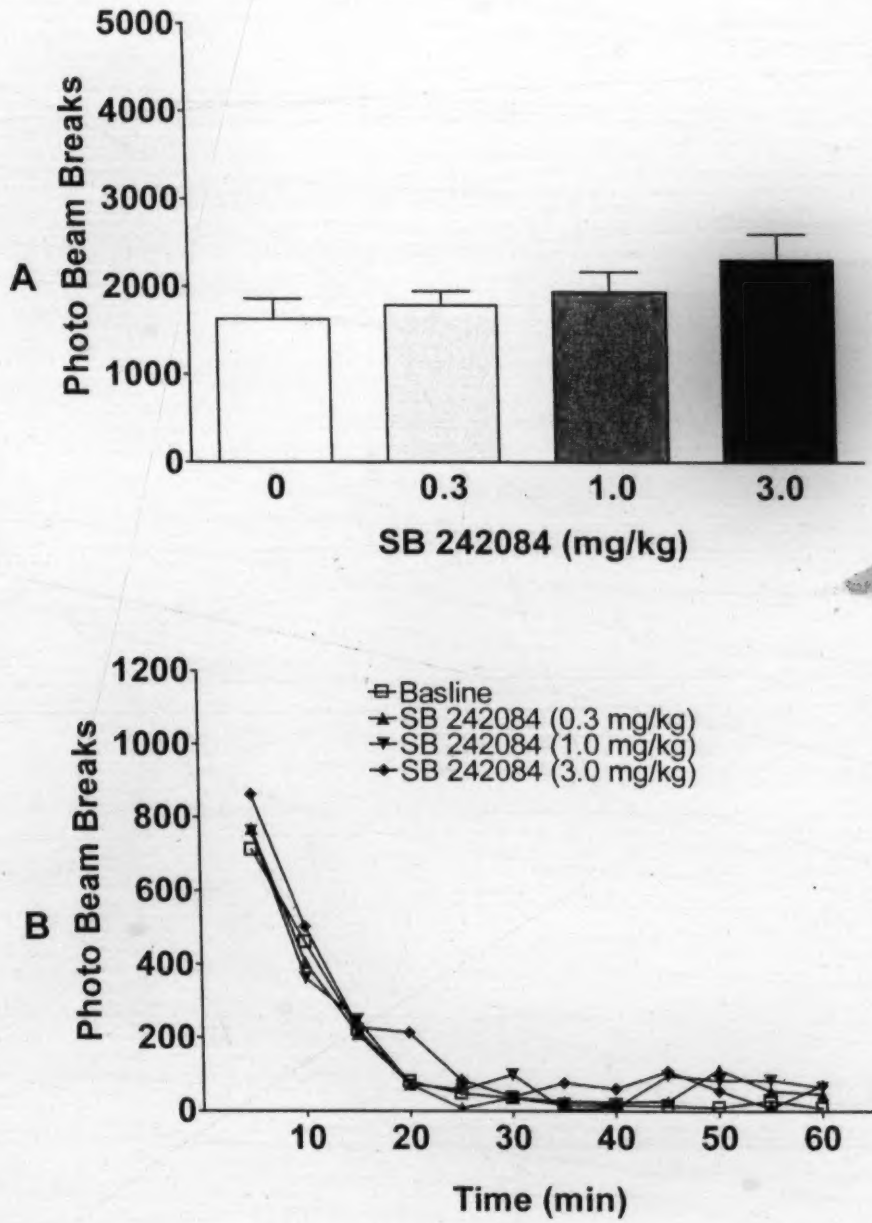
Figure 2.2. Effects of WAY 161503 (1.0 and 3.0 mg/kg) and (+)- AMPH (1.0 mg/kg) with unbiased (A) and biased (B) place conditioning procedures. Data shown are means ± S.E.M. * Significant $P<0.05$.



Experiment 2: Effect of systemic SB 242084 on locomotor activity

Two-way repeated measures ANOVA (dose x time) revealed that there was no significant effect of the 5-HT_{2C} receptor antagonist SB 242084 (0.3-3.0 mg/kg) [$F(2.2, 15.6) = 2.262$, $P>0.05$], there was a significant effect of time [$F(3.1, 21.3) = 87.582$, $P<0.05$], but no significant (SB 242084 x time) interaction [$F(5.3, 36.9) = 0.843$, $P>0.05$], (Figure 2.3A). The lack of effect of SB 242084 on locomotor activity over the 60 min test period is illustrated in Figure 2.3B.

Figure 2.3. Lack of effect of SB 242084 (0.03 – 3.0 mg/kg) on total locomotor activity (A) Data shown are means ± S.E.M. The local time course of changes in horizontal locomotor activity over 60 min (B) are shown as means (SEM were omitted for clarity).

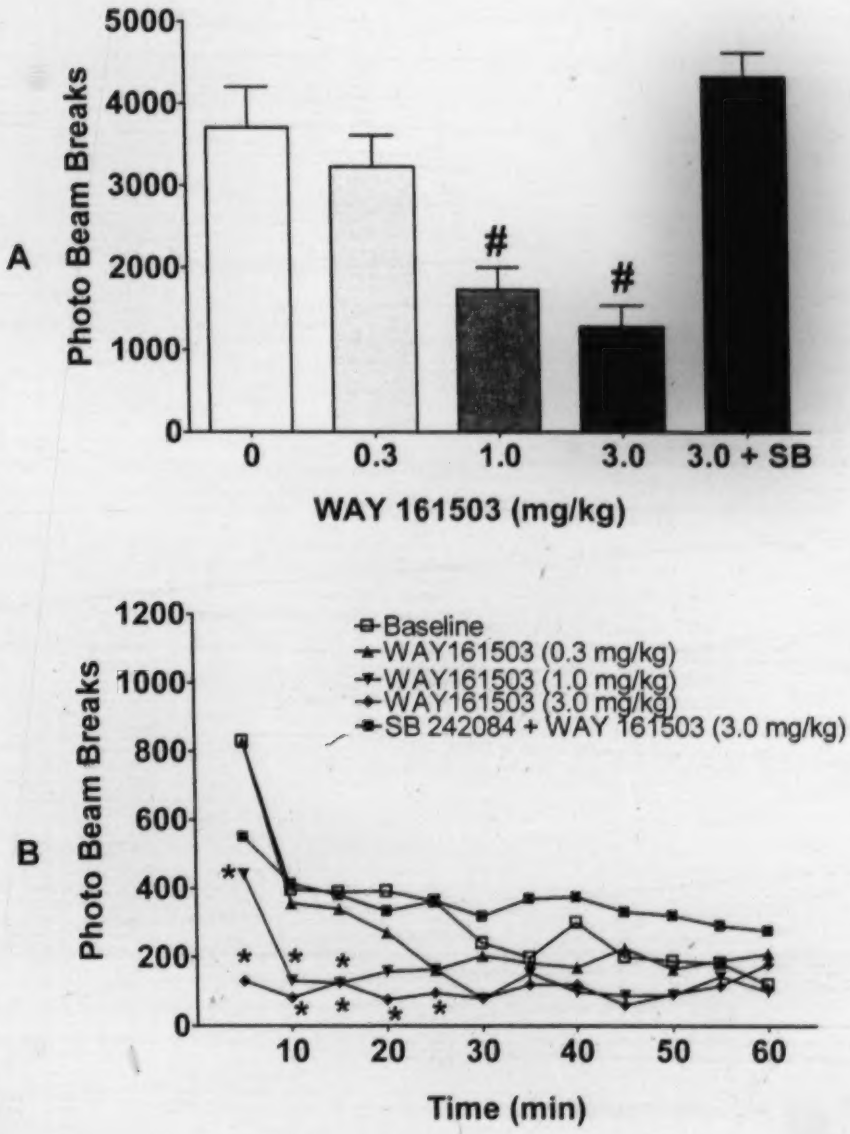


Experiment 3: Effect of Systemic WAY 161503 and SB 242084 on locomotor activity

WAY 161503 decreased spontaneous locomotor activity [$F(1.9, 13.2) = 28.435$, $P<0.05$]. There was a significant effect of time [$F(4.4, 30.8) = 34.931$, $P<0.05$] and a significant (WAY 161503 x time) interaction [$F(5.4, 38.1) = 3.787$, $P<0.05$] (Figure 2.4A).

Since there was a significant interaction, one-way repeated measures ANOVA was performed for each time period. Following a significant one-way ANOVA at a time period, a Newman-Keul's post hoc test ($\alpha=0.05$) was performed. This time course analysis revealed that, at 1.0 mg/kg WAY 161503 reduced locomotor activity during the first 15 min of testing. At 3.0 mg/kg, WAY 161503 reduced locomotor activity during the first 25 min of testing. Pretreatment with SB 242084 blocked the effects of WAY 161503 (3.0 mg/kg; see Figure 2.4B).

Figure 2.4. Effects of the 5-HT_{2C} receptor agonist WAY 161503 (0.03 – 3.0 mg/kg) and WAY 161503 + the 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity over 60 min (B). Data shown are means ± S.E.M. # Significant effect of WAY 161503, $P<0.05$. * Significant $P<0.05$, relative to control.



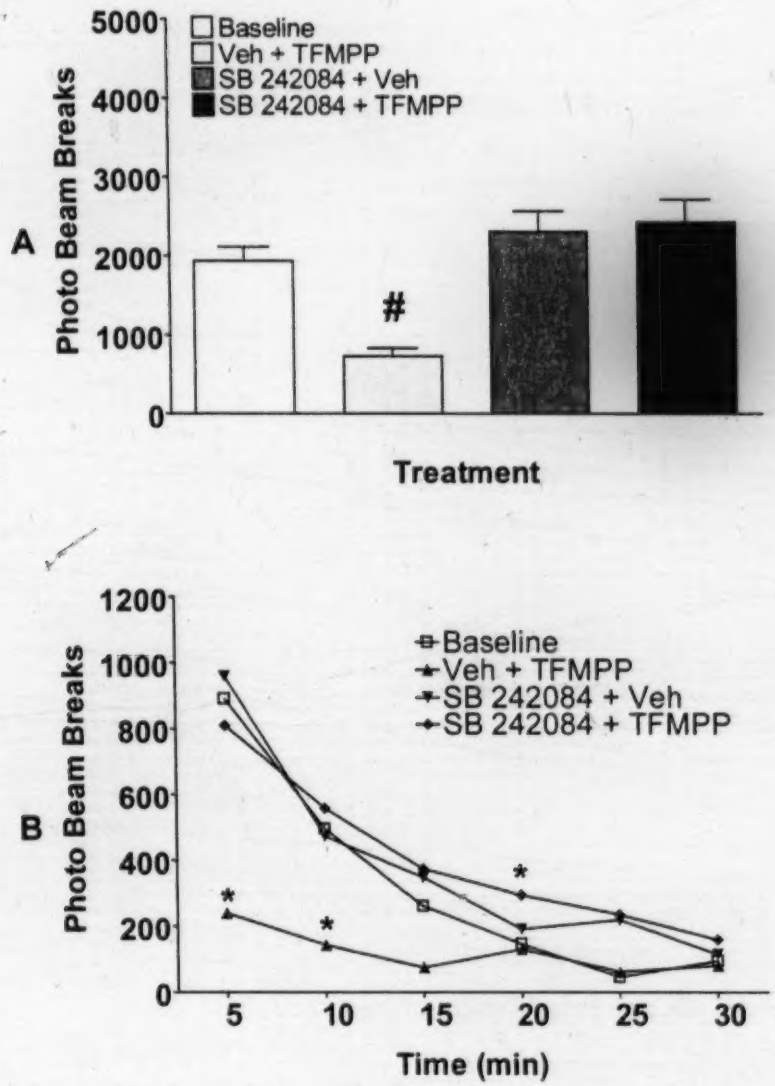
Experiment 4. Effects of 5-HT receptor antagonists on TFMPP-induced locomotor hypoactivity

TFMPP and the 5-HT_{2C} receptor antagonist SB 242084

TFMPP decreased spontaneous locomotor activity [F (1, 7) = 38.178, P<0.05]. There was also a significant effect of the 5-HT_{2C} receptor antagonist SB 242084 [F (1, 7) = 14.652, P< 0.05], and time [F (2.1, 15) = 126.742, P<0.05]. Significant interactions of TFMPP x time [F (2.3, 6.5) = 3.979, P<0.05], SB 242084 x time [F (2.7, 19) = 13.366, P<0.05], SB 242084 x TFMPP [F (1, 7) = 17.199, P< 0.05] and SB 242084 x TFMPP x time [F (3.4, 23.8) = 7.267, P<0.05] were also observed (Figure 2.5A).

TFMPP significantly reduced locomotor activity during the first 10 min. Pretreatment with SB 242084 significantly blocked the decrease in locomotor activity produced by TFMPP for the duration of the test period (Figure 2.5B). Although the two-way repeated measures ANOVA revealed a significant SB 242084 x time interaction, Newman-Keul's post hoc tests failed to reveal any effects of SB 242084 on the time course of changes in locomotor activity measures.

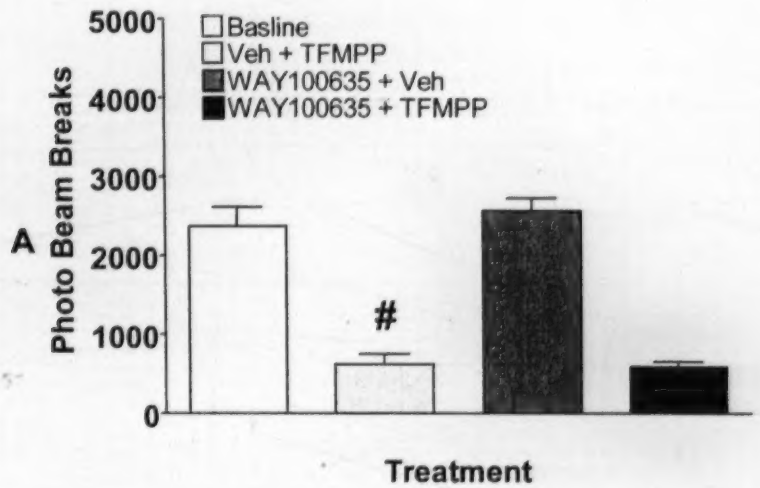
Figure 2.5. Effects of TFMPP (3.0 mg/kg) and SB 242084 (1.0 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 30 min. Data shown are means ± S.E.M. # Significant effect of TFMPP, $P<0.05$. * Significant $P<0.05$, relative to control.

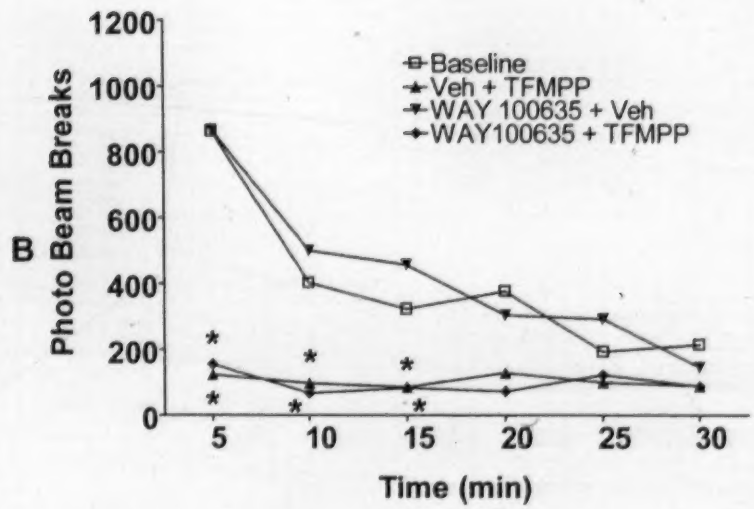


TFMPP and the 5-HT_{1A} receptor antagonist WAY 100635

TFMPP significantly reduced locomotor activity [$F(1, 7) = 39.336$, $P < 0.05$] (Figure 2.6A). There was also a significant effect of time [$F(3.1, 21.9) = 44.433$, $P<0.05$] and a significant TFMPP x time interaction [$F(3.1, 21.6) = 27.907$, $P<0.05$]. The interaction of TFMPP and the 5-HT_{1A} receptor antagonist WAY 100635 [$F(1, 7) = 0.527$, $P > 0.05$] was not significant. WAY 100635 did not block the effects of TFMPP during the testing period. TFMPP and TFMPP + WAY 100635 were significantly different from control during the first 15 min of testing (Figure 2.6B).

Figure 2.6. Effects of TFMPP (3.0 mg/kg) and the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 30 min. Data shown are means ± S.E.M. # Significant effect of TFMPP, $P<0.05$. * Significant $P<0.05$, relative to control.

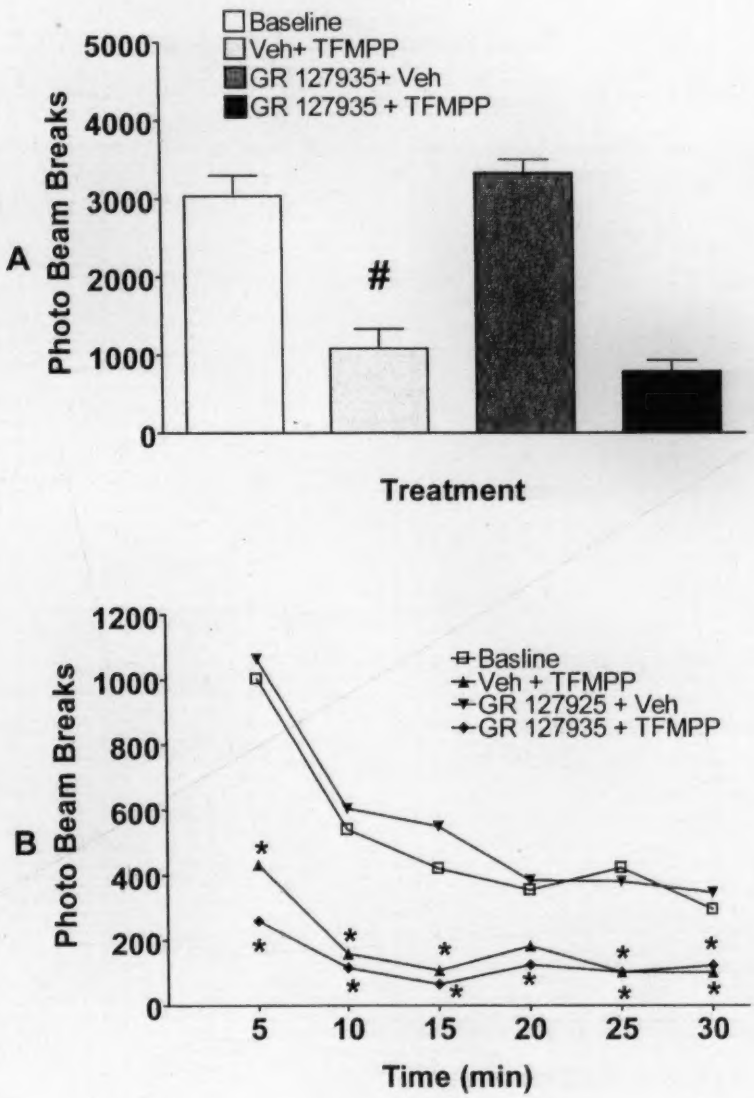




TFMPP and the 5-HT$_{1B/D}$ receptor antagonist GR 127935

TFMPP significantly reduced locomotor activity [$F(1, 7) = 263.244$, $P < 0.05$] (Figure 2.7A). There was also a significant effect of time [$F(2.3, 15.9) = 52.950$, $P<0.05$] and a significant TFMPP x time interaction [$F(2.8, 19.9) = 13.035$, $P<0.05$]. The interaction of TFMPP and the 5-HT$_{1B/D}$ receptor antagonist GR 127935 was not significant [$F(1, 7) = 2.465$, $P>0.05$]. TFMPP significantly reduced locomotor activity for the first 15 min and the last 10 min of testing compared to control. GR 127935 did not block the effects of TFMPP during the 30 min test period (Figure 2.7B).

Figure 2.7. Effects of TFMPP (3.0 mg/kg) and the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 30 min. Data shown are means ± S.E.M. # Significant effect of TFMPP, $P<0.05$. * Significant $P<0.05$, relative to control.



Discussion:

At doses that are behaviourally active (see Figures 2.4-2.7) neither TFMPP nor WAY 161503 induced place conditioning. These drugs, however were effective during this time frame as shown by their effects on locomotor activity. This result is consistent with the observation that the 5-$HT_{1B/2C}$ receptor agonist mCPP failed to induce place conditioning (Rocha et al., 1993). Rocha et al. (1993) have reported that mixed 5-HT receptor antagonists with some affinity for the 5-HT_{2C} receptor (mianserin and eltoprazine) may induce a CPA. That result is difficult to reconcile with the observation that 5-HT_{2C} receptor blockade increases DA release (Di Matteo et al., 1999; Di Matteo et al., 2000b; Di Matteo et al., 2001; Millan et al., 1998; Pozzi et al., 2002). However, mianserin and eltoprazine have high affinities for other receptors (such as the 5-HT_{1B} and α_2 adrenergic receptors). The CPA induced by mianserin and eltoprazine may have been due to actions on multiple 5-HT receptors (Rocha et al., 1993). In contrast, Risinger and Oakes (1996) found that mianserin did not induce a CPA/CPP when administered alone, but did enhance ethanol induced CPP in mice. Although conflicting results have been reported for mianserin in the place conditioning behavioural test (Risinger and Oakes, 1996; Rocha et al., 1993), these may be explained by methodological differences. Risinger and Oakes (1996) used male Swiss-Webster mice, which underwent four drug conditioning trials for a 60 min period. In contrast, Rocha et al. (1993) used male Long-Evans rats, which were conditioned to the drug paired compartment for four trials (30 min each). In addition, Rocha et al. (1993) used a place conditioning apparatus with both visual and tactile cues, whereas Risinger and Oakes (1996) only had tactile cues. The compartments in the place conditioning apparatus used in the present study only differed

in floor texture. Nevertheless, the inclusion of the (+) - AMPH (positive control) in our experiments confirms the validity of the procedures.

The putative 5-HT_{2C} receptor agonist Ro 60-0175 injected systemically or centrally into the VTA, dose-dependently reduced responding for ethanol and for cocaine self-administration (Fletcher et al., 2004; Tomkins et al., 2002). These effects were blocked by pretreatment with the 5-HT_{2C} receptor antagonist SB 242084 (0.5 mg/kg) (Fletcher et al., 2004; Tomkins et al., 2002). This observation is consistent with our present results indicating that 5-HT_{2C} receptor agonists may not exhibit intrinsic rewarding properties, but suggests that activity at these receptors may serve to alter the reinforcing properties of other drugs.

The selective 5-HT_{2C} receptor agonist WAY 161503 induced a dose-dependent decrease in locomotor activity, which was blocked (at the 3.0 mg/kg dose of WAY 161503) by pretreatment with the 5-HT_{2C} receptor antagonist SB 242084. The 5-HT_{2C} receptor antagonist SB 242084 did not significantly alter locomotor activity, although there appeared to be a slight increase in activity as dose increased, which may also be apparent from data presented in other studies (see Martin et al., 2002, Fig 1). TFMPP also induced a marked decrease in locomotor activity, consistent with the earlier findings of Lucki et al. (1989). This effect of TFMPP was blocked by co-administration of SB 242084, but not the specific 5-HT_{1A} receptor antagonist WAY 100635 nor the specific 5-$HT_{1B/D}$ receptor antagonist GR 127935. These results indicate the decrease in locomotor activity induced by TFMPP is mediated by the 5-HT_{2C} receptor, results which are consistent with the actions of other putative 5-HT_{2C} receptor agonists such as Ro 60-0175 (0.5-10 mg/kg), MK 212 (0.31 and 0.62 mg/kg) and mCPP (2.5-7.0 mg/kg)

(Higgins et al., 2001; Kennett et al., 2000; Kennett et al., 1994; Kennett et al., 1997; Lucki et al., 1989; Martin et al., 2002). The present results represent the first specific evidence that the effects of TFMPP on locomotor activity are mediated by the 5-HT_{2C} receptor as suggested by Lucki et al. (1989).

The results of these locomotor activity experiments are also consistent with recent cellular and molecular data that associate 5-HT with mesocorticolimbic DA systems (Di Matteo et al., 2001; Di Matteo et al., 2002). 5-HT_{2C} receptor blockade by SB 242084 increases DA release and basal cell firing in the NAS and VTA respectively (Di Matteo et al., 1999; Di Matteo et al., 2000b; Pozzi et al., 2002), an effect that may be due to inhibition of GABA-containing interneurons in the VTA (Di Matteo et al., 2001). This is supported by work demonstrating that administration of the 5-HT_{2C} receptor agonists Ro 60-0175, mCPP and MK 212 may decrease DA cell firing in the VTA and decrease DA release in the NAS (Di Giovanni et al., 2000; Di Matteo et al., 2000a). As it is hypothesized that increased mesocorticolimbic DA release is reflected by increased locomotion (Dunnett and Robbins, 1992; Mathe et al., 1996), 5-HT_{2C} receptors may regulate or influence DA mediated locomotor activity (Di Matteo et al., 1999).

Further evidence that 5-HT_{2C} receptors affect DA activity comes from studies concerning cocaine- and nicotine- induced changes in locomotor activity. Hyperactivity induced by either cocaine or nicotine may be mediated by the mesocorticolimbic DA system (Fung and Lau, 1988; Koob, 1992; Neisewander et al., 1995; Nisell et al., 1996). It has been demonstrated that Ro 60-0175 injected systemically (0.1-3.0 mg/kg) or centrally (3 and 10 μg/kg) into the VTA reduced cocaine-induced hyperactivity (Fletcher et al., 2004; Grottick et al., 2000). The reduction in cocaine-induced hyperactivity by Ro

60-0175 was blocked by administration of SB 242084 (0.5 mg/kg see Grottick et al. 2000). In addition, Ro 60-0175 dose-dependently blocked nicotine- (0.4 mg/kg) induced hyperactivity. The reduction in nicotine-induced hyperactivity by Ro 60-0175 (1.0 mg/kg) was reversed by pretreatment with SB 242084 [0.5 mg/kg see (Grottick et al., 2001)]. These reports add further evidence for the hypothesis that 5-HT_{2C} receptor activation/blockade may regulate or influence behaviours such as locomotor activity that are regulated by the mesocorticolimbic DA system. In addition to systemic effects of 5-HT_{2C} receptor agonists on locomotor activity, central effects have also been examined. The 5-$HT_{2A/B/C}$ receptor agonist Ro 60-0175 and 5-$HT_{2B/C}$ receptor agonist MK 212 injected into the shell of the NAS, were devoid of effects on locomotor activity, but were able to increase the hyperactivity induced by cocaine (Filip and Cunningham, 2002). In addition, Ro 60-0175 was shown to have no effect on locomotor activity when injected into the VTA (Fletcher et al., 2004). However, when administered systemically these compounds decrease locomotor activity (Higgins et al., 2001; Lucki et al., 1989).

5-HT_{2C} receptors may play a role in the regulation of mesocorticolimbic DA release, effects that may be related to changes in reward/motivational behaviour. The present experiments indicate that selective stimulation of the 5-HT_{2C} receptor may not result in place conditioning. However, 5-HT_{2C} receptor activation did decrease locomotor activity, an effect that was blocked by a selective 5-HT_{2C} receptor antagonist. The present locomotor activity experiments confirm the efficacy of the current drug treatments in the behavioural context. 5-HT_{2C} receptor agonists may modify reinforcing effects of electrical brain stimulation and of self-administered drugs in laboratory animals (Clements et al., ; Fletcher et al., 2004; Tomkins et al., 2002). The lack of a direct

reinforcing effect of 5-HT_{2C} receptor stimulation is illustrated by the current failure of 5-HT_{2C} receptor related drugs to induce place conditioning. This pattern of results indicates that 5-HT_{2C} receptor stimulation may alter rewarding effects of other compounds rather than a directly regulate reinforcement on its own.

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Chapter 3. Aversive stimulus properties of the 5-HT_{2C} receptor agonist WAY 161503 in rats

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Introduction

The DA-containing mesocorticolimbic pathway of the mammalian forebrain may play an important role in the regulation of reward and reinforcement (Bardo, 1998; Ikemoto and Panksepp, 1999; Koob, 1992; McBride et al., 1999; Tzschentke, 1998; Wise and Rompre, 1989; Wise, 2002, 2004). Within this pathway, which projects from the VTA to the NAS and the PFC (Wise and Rompre, 1989; Wise, 2002), 5-HT and DA have significant functional interactions (Di Giovanni et al., 2000; Di Giovanni et al., 2001; Di Matteo et al., 1999, 2000a; Di Matteo et al., 2002). One 5-HT receptor of interest in the context of reward and reinforcement is the 5-HT_{2C} receptor. This receptor is located within the mesocorticolimbic system and is known to affect the activity of DA-containing neurons in this system (Di Giovanni et al., 2000; Di Giovanni et al., 2001; Di Matteo et al., 1999, 2000a; Di Matteo et al., 2001). In addition, activation of this receptor alters behaviours which are mediated by the mesocorticolimbic DA system (Fletcher et al., 2002; Fletcher et al., 2004; Grottick et al., 2000; Grottick et al., 2001; Higgins and Fletcher, 2003; Martin et al., 1998; Rocha et al., 2002).

The inhibitory effect of 5-HT_{2C} receptor stimulation on DA release from mesocorticolimbic terminals is now established (De Deurwaerdere et al., 2004; Di

Giovanni et al., 2000; Di Matteo et al., 1999, 2000a; Di Matteo et al., 2000b; Di Matteo et al., 2001; Di Matteo et al., 2002; Di Matteo et al., 2004; Dremencov et al., 2005; Gobert et al., 2000; Millan et al., 1998; Pierucci et al., 2004; Pozzi et al., 2002). 5-HT_{2C} receptor modulation of DA release may occur by indirect action on DA neurons as 5-HT_{2C} receptor mRNA is not co-localized with tyrosine-hydroxylase (Eberle-Wang et al., 1997). However, 5-HT_{2C} receptor mRNA appears to be located on GABA-containing neurons as 5-HT_{2C} receptor mRNA was expressed within neurons that also were positive for glutamic acid decarboxylase (Eberle-Wang et al., 1997; Serrats et al., 2005). The partial 5-HT_{2C} receptor agonist mCPP was able to increase the firing rate of non-DA neurons in both the substantia nigra and the VTA (Di Giovanni et al., 2001). These authors have suggested that the neurons activated by mCPP were GABA neurons as most non-DA neurons in the VTA contain GABA. In contrast, antagonism of 5-HT_{2C} receptors decreased the inhibitory post-synaptic potentials induced by 5-HT_{2C} receptors on GABA neurons within the DRN (Liu et al., 2000). Based on the evidence that 5-HT_{2C} receptors may be located on GABA-containing neurons, it has been hypothesized that 5-HT_{2C} receptor stimulation activates GABA interneurons in the VTA which synapse on mesocorticolimbic DA neurons, thereby inhibiting DA release in terminal areas of this system (Di Giovanni et al., 2000; Di Giovanni et al., 2001; Di Matteo et al., 2000a; Di Matteo et al., 2001).

Data from behavioural studies on food and drug self-administration as well as ICSS have indicated that an increase in DA release in areas such as the NAS may be associated with an enhancement of reward/reinforcement (Blaha and Phillips, 1990; Blanchard et al., 1993; Hernandez and Hoebel, 1988; Kiyatkin and Stein, 1995; Pettit and

Justice, 1989, 1991; Volkow et al., 2004; Wise, 2004; You et al., 2001). The inhibitory effect of 5-HT_{2C} receptor stimulation on mesocorticolimbic DA release is consistent with an inhibitory role for these receptors in reward or reinforcement (Di Giovanni et al., 2000; Di Matteo et al., 2000a; Higgins and Fletcher, 2003; Tzschentke, 1998). This hypothesis is supported by behavioural studies which indicate that systemic administration of 5-HT_{2C} receptor agonists decrease reward, indicated by increased thresholds for ICSS of the VTA (Clements et al., 2004; Hayes and Greenshaw, 2004), and decreased responding for ethanol, nicotine and cocaine self-administration (Grottick et al., 2000; Grottick et al., 2001; Maurel et al., 1999; Tomkins et al., 2002). In addition, intra-VTA injection of the mixed 5-HT_2 receptor agonist Ro 60-0175 decreased responding for cocaine self-administration (Fletcher et al., 2004).

Although it is well established that DA plays a role in reinforcement, a decrease in DA release is not necessarily associated with a decrease in reinforcement or the presence of an aversion. Previous experiments using selective (Mosher et al., 2005) or non-selective agonists at the 5-HT_{2C} receptor (Mosher et al., 2005; Rocha et al., 1993) were unable to demonstrate place conditioning when animals were tested in a drug-free state. In addition, depletion of DA in the medial PFC by 6-hydroxydopamine lesion failed to abolish cocaine self-administration (Schenk et al., 1991), and mice that are genetically modified so that they are DA-deficient, still display a normal preference for sucrose (Cannon and Bseikri, 2004). In addition, no changes in NAS extracellular DA levels were observed following aversive foot-shock conditioning (Levita et al., 2002).

Recent work from our laboratory has shown that either selective or non-selective stimulation of the 5-HT_{2C} receptor does not induce place conditioning when animals are

tested in a drug-free state; however (+) -AMPH did induce a CPP under these conditions (Mosher et al., 2005). The lack of place conditioning following 5-HT_{2C} receptor activation is in agreement with Rocha et al. (1993) using the non-specific 5-HT_{2C} receptor agonist mCPP. As previous studies have failed to induce place conditioning following stimulation of the 5-HT_{2C} receptor when the animals were tested in a drug-free state, the current study investigated whether activation of this receptor would induce place conditioning when the animals were tested in a drugged state. State dependency refers to a behaviour or response which was acquired while in a given state (e.g. drugged state) and can only be expressed if the animal is tested in the same state (Tzschentke, 1998). Previous studies have shown that compounds such as diazepam, cocaine, morphine, naloxone and lithium are able to induce place conditioning when animals are tested in a drugged state (Herzig and Schmidt, 2004; Mucha and Iversen, 1984; Nomikos and Spyraki, 1988; Oberling et al., 1993; Olmstead and Franklin, 1997; Sakoori and Murphy, 2005; Spyraki et al., 1985).

Despite the observation that 5-HT_{2C} receptor stimulation has effects on primary reinforcement, as shown by the studies described above, the lack of place conditioning following administration of a 5-HT_{2C} receptor agonist raises the question of whether 5-HT_{2C} receptor stimulation is an effective stimulus for the induction of conditioned reinforcement. The CTA behavioural test is very sensitive to effects of drugs in this context (Goudie, 1986). With respect to 5-HT-related compounds, CTA may be elicited by 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2B}, and 5-HT_{2C} receptor stimulation and 5-HT_3 receptor antagonism (Arnold et al., 1995; Berendsen and Broekkamp, 1999; De Vry et al., 2000; Guitton and Dudai, 2004). Based on those findings, the present study also included an

assessment of the ability of the 5-HT_{2C} receptor agonist WAY 161503 to induce a CTA to saccharin. The induction of a CTA appears to be independent of the observation of place conditioning as the partial 5-HT_{2C} receptor agonists mCPP and TFMPP failed to induce place conditioning (Mosher et al., 2005; Rocha et al., 1993) but did induce a CTA to saccharin (De Vry et al., 2000). It was hypothesized that 5-HT_{2C} receptor activation may induce state-dependent place conditioning and a CTA to saccharin; (+) -AMPH was used as a positive control to demonstrate the efficacy of the place conditioning and CTA procedures.

Materials and Methods

Animals

Male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 150-250g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights on 0700 h -1900 h). Food was freely available in the home cages. Water was freely accessible during place conditioning experiments; however, throughout the CTA experiment, the animals were restricted to 30 min/day access to water). The care and use of the animals complied with the guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Place Conditioning

Apparatus: The place conditioning apparatus (I. Halvorson System Design, Phoenix, AZ, USA) consisted of a rectangular Plexiglas box divided into two compartments (30 cm L x 30 cm W x 25 cm H). The compartments differed only in floor texture: 14 horizontal bars positioned 1.25 cm apart compared with 1-cm square grate wire flooring. The compartments were separated by a white plastic divider, which contained a tunnel (7.5 cm long) allowing access to both compartments that could be obstructed with removable doors during conditioning.

Procedure: The procedure consisted of three phases. *Phase1* (Pre-Conditioning): The animals (n=8 per group) were habituated to the place conditioning apparatus for three consecutive days, during which the animals had free access to both compartments for 15 min. On the third day of pre-conditioning, the amount of time spent in each compartment was recorded to the nearest second. The animals were randomly assigned to each drug group and adjusted if necessary to equalize baseline compartment preference between groups and within each group to achieve an unbiased place conditioning design. *Phase2* (Conditioning): On alternating days, the animals received drug or vehicle treatment and were confined to the drug-paired or vehicle-paired compartment for 30 min. The animals were conditioned for six consecutive days. *Phase3* (Post-Conditioning): During retention testing, the animals received an injection of the drug which they were conditioned to on the first day of post-conditioning and were then placed in the apparatus with free access to both compartments for 15 min. This procedure was repeated for two more days with the exception that the animals were tested in a drug-free state. The amount of time spent in each compartment was recorded to the nearest second.

All testing took place under red light during the light phase of the light/dark cycle. The place conditioning apparatus was cleaned between each animal with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia).

Conditioned Taste Aversion Procedure

Measurement of baseline water consumption

Standard drinking bottles, each equipped with a double ball bearing to prevent leakage were used. For eight days, the rats were placed on restricted water access; the animals were given access to water for 30 min/day (1130 am - 1200 pm) in order to determine baseline water consumption. Water bottles were filled on the day prior to each drinking session in order to allow the temperature of the water to equilibrate to room temperature. During the drinking session, each rat was given access to two bottles of water for a 30 min period. Bottle positions were switched after 15 min to prevent position bias. Before and after each drinking session, every water bottle was weighed to the nearest 0.1 g. Water consumption was calculated to the nearest 0.1 ml by taking the difference between the weight of the bottles before and after drinking.

Conditioning and assessment of saccharin preference

On conditioning days (9-11) the procedure followed was the same as described for the measurement of baseline water consumption except the rats had access to two bottles of 0.1% saccharin solution. The animals were randomly assigned to one of three treatment groups (n= 8 per group) and received an injection of either saline, (+)-AMPH or WAY 161503 immediately following the end of the drinking session. All treatment groups were given access to one bottle of water for a 30 min period (4:30-5:00 pm) to

ensure that the rats that had reduced fluid intake due to either neophobia (conditioning day 9) or had developed a conditioned aversion to the saccharin solution (conditioning days 10 and 11) would have a sufficient daily intake of fluid.

Extinction two-bottle choice test

Extinction testing took place over three days; this procedure has been used in our laboratory previously and is based on Arnold et al. (1995). During extinction testing (days 12-14), subjects were given a two-bottle choice test with access to one bottle of saccharin solution and one bottle of water for 30 min (11:30 am – 12:00 pm). The same procedure and method of measurement were used as described for fluid baseline consumption of the water and saccharin solution.

Drugs

The 5-HT_{2C} receptor agonist WAY 161503 ·HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride] with a 2000-fold selectivity over the 5-HT_{2A} receptor (Rosenzweig-Lipson et al., 2000) was purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+)-AMPH sulphate was purchased from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). WAY 161503 was dissolved in doubled distilled water, and (+)-AMPH was dissolved in 0.9% saline. All drugs were injected s.c. in a volume of 1.0 ml/kg. Drug doses are expressed as free-base.

Drug Treatment

Experiment 1: Effect of WAY 161503 on place conditioning

The effects on place conditioning of WAY 161503 (1.0 and 3.0 mg/kg, s.c., 10 min prior) were investigated. A positive control group which received (+)-AMPH (1.0 mg/kg, s.c., 10 min prior) was included. All treatment groups (n=8 per group) received three injections of their respective drug treatment and vehicle on alternating days. Animals were then tested in a drugged state (post-conditioning day 1) to determine if there were state-dependent reinforcing effects of the drugs on place-conditioning.

Experiment 2: Effects of WAY 161503 on conditioned taste aversion

The animals were randomly divided into each drug group (n=8 per group) to determine the effects of WAY 161503 (3.0 mg/kg, s.c.) on CTA. This dose of WAY 161503 was chosen as it has previously been shown to induce behavioural changes in a locomotor activity test (Mosher et al., 2005). A control group which received saline (0.9% saline solution is used as a control wherever possible) and a positive control group which received (+)-AMPH (2.5 mg/kg, s.c.) were also included in the study.

Statistical Analyses

Experimental effects on place conditioning were determined by paired sample t-tests to compare time spent in the drug-paired side on pre-conditioning day 3 and post-conditioning day 1 ($P \leq 0.05$, n=8 per group). Following a significant t-test, the animals were then tested for two more days in a drug-free state and analyzed using a one-way repeated measures ANOVA [pre-conditioning day 3 and post conditioning days 1, 2 and

3]. The Newman-Keul's post hoc test was used where appropriate to compare pre-conditioning day 3 to post-conditioning days (α=0.05).

Experimental effects of saccharin intake on conditioning days with paired injections of either saline, WAY 161503 or (+)-AMPH were determined using a two-way (saccharin exposure day x drug treatment) repeated measures ANOVA; drug treatment group was a between subjects factor. Where appropriate, analysis of saccharin exposure day was analyzed using one-way repeated measures ANOVA across all drug groups. A significant F ratio ($P \leq 0.05$) on a single saccharin exposure day was followed by comparison of each drug condition to control using Newman-Keul's post hoc tests (α=0.05). Results from the test days (extinction/retention days) were analyzed using a two-way (test day x drug treatment) repeated measures ANOVA; drug treatment was a between subjects factor. All statistical analyses were completed using SPSS statistical software (SPSS 11.5, SPSS Inc. Chicago, U.S.A.).

Results

Experiment 1: Effect of WAY 161503 on place conditioning

WAY 161503 (3.0 mg/kg) [$t(7) = 2.49$, $P < 0.05$], but not WAY 161503 (1.0 mg/kg) [$t(7) = 0.79$, $P > 0.05$] induced a state-dependent CPA. Under these conditions (+)-AMPH induced a significant CPP [$t(7) = -3.56$, $P < 0.05$] (Figure 3.1). A one-way repeated measures ANOVA (pre-conditioning day 3, post-conditioning days 1-3) was performed on groups conditioned to WAY 161503 (3.0 mg/kg) and (+)-AMPH. Analysis showed that there was a significant effect of test day in both WAY 161503 (3.0 mg/kg)- [$F(3,21) = 4.97$, $P<0.05$] and (+)-AMPH- [$F(3,21) = 6.483$, $P<0.05$] treated animals.

Newman-Keul's post hoc test revealed that the CPA induced by WAY161503 and the CPP induced by (+)-AMPH was not present on post-conditioning days 2 and 3 (Figure 3.2A and 3.2B respectively).

Figure 3.1. State-dependent effects of WAY 161503 (1.0 and 3.0 mg/kg) and (+)-AMPH (1.0 mg/kg) on place conditioning using an unbiased design. Data shown are means ± S.E.M. * Significant $P<0.05$.

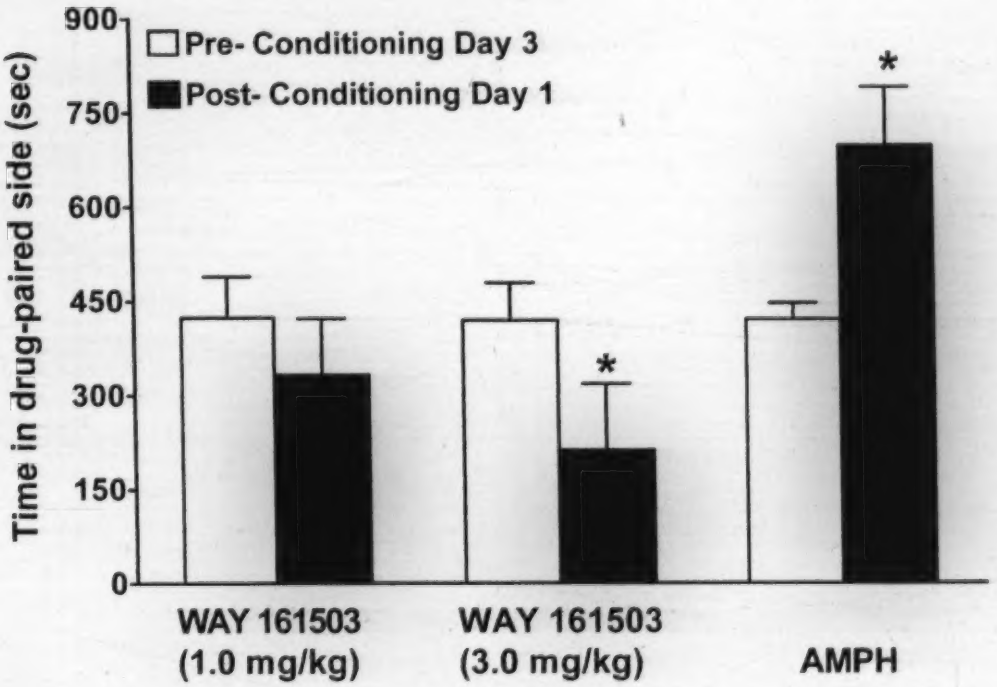
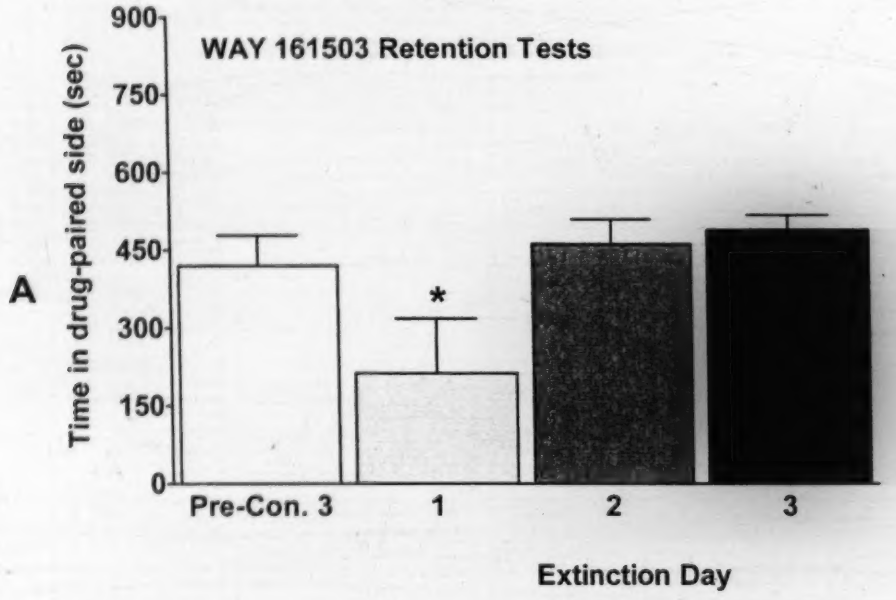
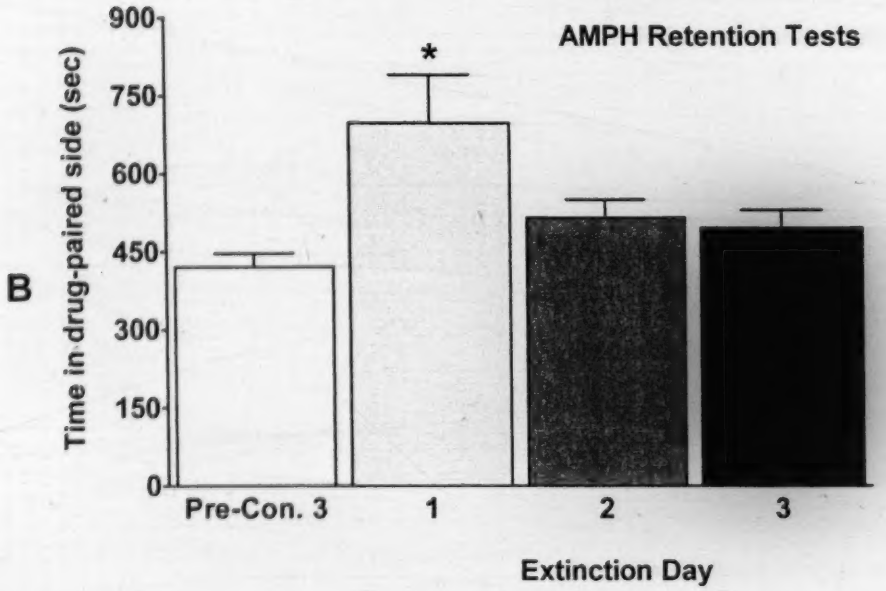


Figure 3.2. Extinction testing for WAY 161503 (3.0 mg/kg) (A) and (+)-AMPH (1.0 mg/kg) (B). Data shown are means ± S.E.M. * Significant P<0.05.





Experiment 2: Effects of WAY 161503 on conditioned taste aversion

Saccharin Conditioning

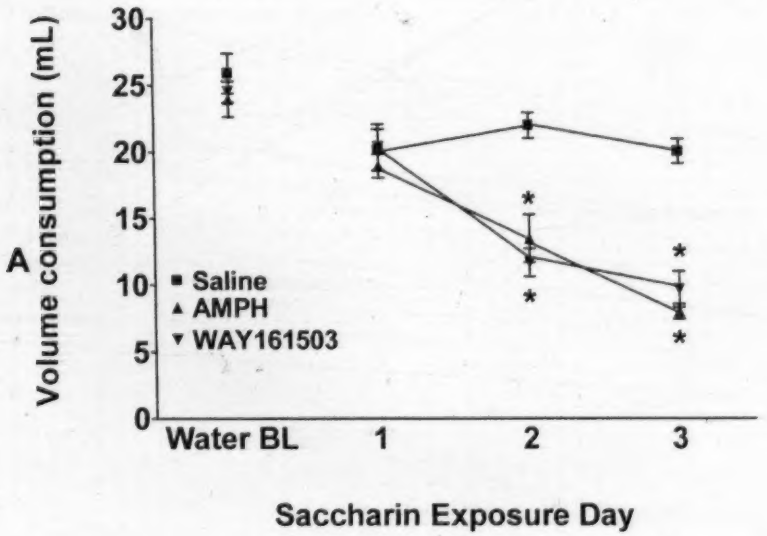
There was a significant effect of saccharin exposure day [$F(1.88, 39.66)=24.919$, $P<0.05$], a significant effect of treatment group [$F(2,21)=30.17$, $P<0.05$] and a significant (saccharin exposure day x treatment group) interaction [$F(3.77,39.66)=7.53$, $P<0.05$]. Since there was a significant interaction, a one-way ANOVA was performed for each saccharin exposure day. Following a significant one-way ANOVA on a saccharin exposure day, a Newman-Keul's post hoc test was performed. The analysis of the saccharin exposure days revealed that on the first (pre-drug) day of saccharin exposure, there was no difference in saccharin consumption between the groups. However, on the second [$F(2,21) = 17.39$, $P<0.05$] and third [$F(2,21)=43.15$, $P<0.05$) days of saccharin exposure WAY 161503 and (+)- AMPH treated animals consumed significantly less saccharin solution than saline treated animals (Figure 3.3A).

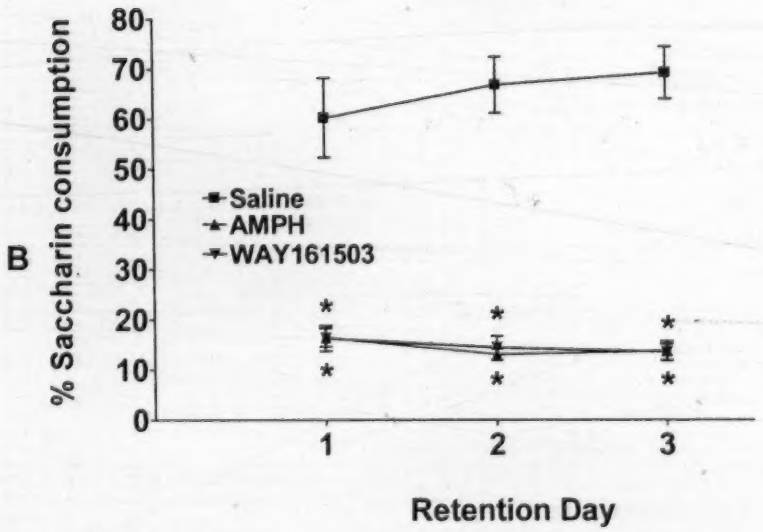
Assessment of Saccharin Preference

Across extinction trials (% saccharin intake), there was a significant effect of treatment group [$F(2,21)=107.44$, $P<0.05$], but no effect of test day (extinction trial) or significant interaction (test day x treatment group). As there was no significant effect of test day (extinction trial), a one-way ANOVA collapsed across test days was then performed with drug treatment as a between subjects factor [$F(2,69)=167.747$, $P<0.05$]. Following a significant F ratio, comparison of each drug condition to vehicle using Newman-Keul's post hoc test was performed. Post hoc tests revealed that WAY 161503- and (+)-AMPH- treated animals had a decreased % saccharin intake compared to saline treated animals (Figure 3.3B). A one-way ANOVA between treatment groups indicated

that during the three extinction trial days, there was no significant difference in the volume of liquid consumed between the three drug treatment groups.

Figure 3.3. Effects of WAY 161503 (3.0 mg/kg), (+)-AMPH (2.5 mg/kg) and saline on (A) saccharin intake (mL) across the three conditioning days and baseline water consumption (mL) (B) % saccharin intake. Data shown are means ± S.E.M.





Discussion

Previous experiments using selective (Mosher et al., 2005) or non-selective agonists at the 5-HT_{2C} receptor (Mosher et al., 2005; Rocha et al., 1993) were unable to demonstrate place conditioning when the animals were tested in a drug-free state. The results of the present study are, however, consistent with reward-decreasing effects of activation of this receptor, as administration of the selective 5-HT_{2C} receptor agonist WAY 161503 induced a state-dependent CPA. In previous experiments examining the effects of 5-HT_{2C} receptor stimulation (Mosher et al., 2005; Rocha et al., 1993), the animals received no injections on post-conditioning day 1, and no place conditioning was observed. These studies indicate that the CPA observed in the present study is related to state-dependent effects and not to the fact that it was observed on the first post-conditioning day. Increases in DA release can be associated with an enhancement of rewarding effects (or perhaps enhancement of incentive salience). Although, it should be noted that an increase in DA release may not always be associated with positive or rewarding effects (i.e. stress); conversely, a decrease in mesocorticolimbic DA release may not be aversive (Cabib and Puglisi-Allegra, 1996; Imperato et al., 1992; Kehoe et al., 1998). Nonetheless, it is possible that the observed state-dependent CPA may be related to a reduction in DA transmission. DA D_2 receptor antagonists that decrease DA transmission may also induce CPAs when injected alone as well as reduce reinforcing effects of other drugs. For example, intra-septal but not intra-amygdala injection of the DA D_2 receptor antagonist sulpiride induced a CPA (Karami et al., 2003; Rezayof et al., 2002). Either intra-septal or intra-amygdala administration of sulpiride may reduce the acquisition of morphine-induced CPP (Karami et al., 2003; Rezayof et al., 2002). The

expression of morphine induced CPP was also attenuated by intra-amygdala injection of sulpiride (Rezayof et al., 2002). Intra-NAS injections or systemic administration of sulpiride blocked the expression and acquisition respectively of the CPP induced by AMPH (Hiroi and White, 1991). In contrast, the CPP induced by cocaine was unaffected by sulpiride administration (Cervo and Samanin, 1995). Although a reduction in DA levels in the mesocorticolimbic system may account for the induction of a CPA by WAY 161503, decreases in DA release do not necessarily correlate with a decrease in reward/reinforcement (Cannon and Bseikri, 2004; Levita et al., 2002; Schenk et al., 1991). More recently, it has been proposed that DA cell firing may encode changes in reward expectancy or cue recognition rather than the reward itself (Schultz, 2006).

McClure et al. (2003) proposed a computational model in which changes in DA transmission encode the incentive salience of the reward rather than the reward itself. This model has been supported by behavioural studies linking changes in DA neuronal cell firing and encoding of information about the expectation of reward using environmental cues. In animals trained to discriminate between stimuli that predicted the presence/ absence or magnitude of a reward, DA neuronal cell firing increased or decreased following the onset of the conditioned stimuli (Tobler et al., 2003; Tobler et al., 2005). DA D_1 receptor knock-out mice displayed an impairment in the pre-reward excitatory response in the NAS and an impairment in the acquisition of a place learning task (a spatial memory task) compared to wildtype control mice (Tran et al., 2005). These results indicate a role for NAS DA D_1 receptors in the acquisition of spatial memory tasks associated with reward (Tran et al., 2005). The above studies suggest that DA neuronal cell firing may encode the incentive value or the prediction of

reward/reinforcement. Consequently, it is possible that compounds which reduce DA neuronal cell firing may induce aversive reinforcement in the place conditioning behavioural test. Stimulation of 5-HT_{2C} receptors is known to decrease the firing of DA neurons (Di Giovanni et al., 2001; Di Matteo et al., 2000b; Gobert et al., 2000). Therefore, activation of 5-HT_{2C} receptors may lead to an association of the environment with the prediction of a decrease in reinforcement and lead to the induction of a CPA.

In the place conditioning behavioural test, evidence of associative learning in the animal is used to indicate positive or negative reinforcing effects of drugs. In other animal models of associative learning such as classically conditioned changes in the nictitating membrane response in rabbits, the conditioned avoidance response in rats and autoshaping task in rats, partial 5-HT_{2C} receptor agonists were found to alter learning and memory processes (Alhaider et al., 1993; Gimpl et al., 1979; Harvey et al., 1982; Harvey et al., 1988; Harvey, 1996; Meneses and Hong, 1997a, b; Meneses, 2002, 2003; Romano et al., 1991; Romano and Harvey, 1994; Schindler et al., 1985; Siegel and Freedman, 1988; Welsh et al., 1998a; Welsh et al., 1998b). At least one study has shown that activation of 5-HT_{2C} receptors may be an effective discriminative stimulus using a fixed ratio, food reinforced task (Dekeyne et al., 1999). In addition, there is evidence for a role of 5-HT_{2C} receptors in mediating the discriminative stimulus effects of cocaine (Callahan and Cunningham, 1995; Filip and Cunningham, 2002, 2003; Frankel and Cunningham, 2004), DOM (Eckler et al., 2004), citalopram (Grant and Colombo, 1993; Grant et al., 1997; Millan et al., 1999) and ethanol (Maurel et al., 1998).

In addition to altering learning and memory, 5-HT receptor ligands may induce changes in sensorimotor integration. For example, the mixed 5-HT receptor agonists

DOI (Farid et al., 2000; Sipes and Geyer, 1994) and RU 24969, but not mCPP, disrupted pre-pulse inhibition in rats (Sipes and Geyer, 1994). Furthermore, increased acoustic startle following administration of mescaline was reversed by pretreatment with 5-HT_2 receptor antagonists (Davis, 1987). 5-HT also decreased acoustic startle when injected into the lateral cerebral ventricles, but increased this measure when injected into spinal cord (Davis et al., 1980). 5-HT_{1A} receptor agonists may impair sensorimotor performance in the open field as measured by hindlimb abduction and exorotation (Demeulemeester et al., 2001) and increase thigmotaxic (i.e. swimming around the perimeter of the pool) behaviour in the Morris water maze (Luttgen et al., 2005). Drugs that elicit release of 5-HT may induce disruption of auditory and visual pre-pulse inhibition (Kehne et al., 1996). These studies indicate clearly that activation of some 5-HT receptors may disrupt associative learning and sensorimotor integration.

It is known that 5-HT_{2C} receptor activation alters DA function in the mesocorticolimbic system (Di Giovanni et al., 2000; Di Matteo et al., 2001; Gobert et al., 2000) and that compounds which act at 5-HT receptors may alter the sensory environment of the animal. In addition, alterations in DA function may encode reward prediction based on environmental cues. During conditioning, the animal may experience both a change in DA activity as well as an altered sensory environment compared to when the animals is in a drug-free state. Thus, if the animals are tested in a drug-free state, place conditioning may not be observed due to the absence of the "sensory" environment that may have been present during conditioning. In the absence of the conditioning cues induced by the 5-HT_{2C} receptor agonist, the conditioned and unconditioned compartments of the apparatus may both be equally preferable to the

animals similar to that observed during pre-conditioning and thus place conditioning may not be observed. Therefore, the state of the animal may be related to the expectation/observation of reward. This explanation is speculative and future studies will need to be undertaken to address this hypothesis.

Activation of 5-HT_{2C} receptors by the mixed 5-HT receptor agonists MK 212, TFMPP and mCPP also exert anxiety-inducing or "anxiogenic" effects (de Mello Cruz et al., 2005; Guitton and Dudai, 2004; Kennett et al., 1989), a factor that could account for the induction of a CPA. Nevertheless, those effects may be due to non-specific actions of these drugs as 5-HT_{2C} receptor stimulation by Ro 60-0175 did not induce clear anxiogenic effects in the rat social interaction test (Kennett et al., 2000). In addition, drugs which are believed to be anxiety-inducing such as $GABA_A$ receptor antagonists may induce CPAs when animals are tested in a drug-free state (Thielen and Shekhar, 2002). If the anxiogenic properties of the drug were sufficient to induce a CPA, such an effect would be observed when the animal is tested in a drug-free state. The current place conditioning experiment did not include a control group that received a vehicle injection on post-conditioning day 1; therefore the lack of effect on retention days 2 and 3 may be due to either extinction or state dependency. In a previous experiment carried out in our laboratory using a 5-HT receptor agonist, an injection on post-conditioning day 1 did not induce a CPA; however, when animals were tested in a drug-free state, a CPA was observed (unpublished results). That result indicates that the action of receiving an injection on post-conditioning day 1 may be insufficient to induce place conditioning and supports the proposal that the CPA observed in the present study is likely due to state-dependent effects of the drug.

In view of the prior failure to demonstrate place conditioning using 5-HT_{2C} receptor agonists as unconditioned stimuli (Mosher et al., 2005; Rocha et al., 1993), a CTA experiment was also performed to determine if activation of the 5-HT_{2C} receptor could act as a functionally aversive stimulus properties, the behavioural consequence of which would be evident in the drug-free state. The CTA is a behavioural test that is extremely sensitive for measuring reinforcing stimulus properties of drugs (Goudie, 1986).

Although it is an aversive conditioning paradigm, many drugs of abuse (evinced by effects in behavioural tests such as self-administration, ICSS and place conditioning), including AMPH, cocaine, nicotine and morphine, also induce CTA learning (Etscorn et al., 1987; Goudie et al., 1978; Greenshaw and Dourish, 1984; Kumar et al., 1983; LeBlanc and Cappell, 1975; Miller and Miller, 1983; Pilcher and Stolerman, 1976; Van der Kooy and Phillips, 1977). From an evolutionary perspective it has been proposed that CTA learning is related to avoidance of noxious substances in relation to ingestion (Goudie, 1986; Mediavilla et al., 2005). Thus, in this behavioural test, virtually any stimulus that induces a long term change in the affective state of the rat (including drugs of abuse) may lead to subsequent avoidance of the flavour [conditioned stimulus] that precedes that state [unconditioned stimulus] (Bureš and Burešová, 1982).

The observation of a CTA following pairing of the saccharin solution with WAY 161503 further supports the notion that activation of 5-HT_{2C} receptors induces aversive reinforcing effects. However, in contrast to place conditioning, the CTA induced by 5-HT_{2C} receptor activation was not state-dependent. Differential effects (i.e. state dependency) in these two behavioural tests may be due to activation of different brain

systems in each of these aversive conditioning contexts. Following lesions of the parabrachial nucleus, place conditioning effects induced by morphine and lithium chloride (Bechara et al., 1993; Reilly et al., 1993) as well as ICSS of the medial forebrain bundle (Waraczynski and Shizgal, 1995) were intact, whereas CTA learning was disrupted (Bechara et al., 1993; Reilly et al., 1993). In addition, aversive place conditioning effects of morphine withdrawal in morphine-dependent rats was still observed following lateral parabrachial nucleus lesions (Nader et al., 1996). These studies suggest a role for two separate brain systems mediating the reinforcing effects of place conditioning and CTA. As CTA learning may have evolutionary importance, it is possible that this phenomenon may not be state-dependent, and that caudal regions of the brain such as the brainstem may be responsible for the development of CTA (Bureš Burešová, 1989; Bureš et al., 1991; Reilly, 1999). It appears that separate brain areas mediate place conditioning and CTA learning. As more caudal brain regions are thought to be responsible for CTA learning, it is possible that phylogenetically younger regions are necessary for place conditioning learning. Although the proposal of separate brain regions mediating CTA and place conditioning learning is speculative, it is consistent with a greater dependency on higher cortical function involved in the encoding of external environmental cues (Bechara et al., 1993; Curtis and D'Esposito, 2004), but not gustatory avoidance learning (Bureš et al., 1991).

As 5-HT receptor activation may have effects on sensorimotor integration as well as learning and memory (Altman and Normile, 1988; Harvey, 1996; Meneses and Hong, 1997a; Meneses, 2003) it is possible that these factors are responsible for the state-

dependent nature of the CPA induced by WAY 161503. Further place conditioning studies with 5-HT receptor related compounds should include a test for state-dependency.

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Chapter 4. Effects of RU 24969 and CP 94253 on place conditioning and locomotor activity in rats

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Introduction

The 5-HT_{1B} receptor is a G-protein coupled receptor that may exist as a terminal autoreceptor on 5-HT neurons (Barnes and Sharp, 1999; Hoyer et al., 2002) or as a heteroceptor on neurons containing acetylcholine (Cassel et al., 1995), glutamate (Boeijinga and Boddeke, 1996) or GABA (Bruinvels et al., 1994; Chadha et al., 2000; Yan and Yan, 2001a). In the brain, the highest densities of 5-HT_{1B} receptor binding sites are located in the dorsal subiculum, globus pallidus, ventral pallidum and the substantia nigra (Bruinvels et al., 1993; Pazos and Palacios, 1985; Sari, 2004). In addition, there is also a moderate density of 5-HT_{1B} receptor binding sites in the VTA (Bruinvels et al., 1993), where they appear to be heteroceptors on the terminals of GABA neurons (Bruinvels et al., 1994; Yan and Yan, 2001a). Systemic administration of the mixed 5-HT (5-$HT_{1A/B/2C}$) receptor agonist RU 24969 may increase DA release in the NAS (Boulenguez et al., 1996). Infusion of 5-HT_{1B} receptor agonists into the VTA may also elevate extracellular DA concentrations within the VTA and NAS; administration of 5-HT_{1B} receptor agonists into the NAS or PFC may increase DA levels in each area respectively (Iyer and Bradberry, 1996; O'Dell and Parsons, 2004; Yan and Yan, 2001b; Yan et al., 2004). In addition, 5-HT_{1B} receptor stimulation may enhance the increase in

mesocorticolimbic DA levels induced by administration of other compounds such as ethanol (Yan et al., 2005) and cocaine (O'Dell and Parsons, 2004; Parsons et al., 1999). In contrast to the effect on mesocorticolimbic DA levels, activation of 5-HT_{1B} receptors in the VTA decreases GABA concentrations in this area (O'Dell and Parsons, 2004; Parsons et al., 1999; Yan and Yan, 2001a; Yan et al., 2004). Several researchers have proposed that 5-HT_{1B} receptor stimulation may increase DA concentrations by altering GABAergic inhibition in the mesocorticolimbic system (Johnson et al., 1992; Parsons et al., 1999; Yan and Yan, 2001a; Yan et al., 2004). As mesocorticolimbic DA transmission may play a role in reinforcement (Ikemoto and Panksepp, 1999; Pierce and Kumaresan, 2006; Tzschentke, 1998; Wise, 2004) and locomotor activity (Di Chiara and Imperato, 1988; Imperato et al., 1986), activation 5-HT_{1B} receptors may alter behaviours in that context.

Few studies have reported effects of 5-HT_{1B} receptor stimulation in behavioural tests used to measure reward and reinforcement. Activation of the 5-HT_{1B} receptor may induce aversive effects as measured using the ICSS, drug self-administration and place conditioning behavioural tests. For example, systemic administration of the mixed 5-HT receptor agonist RU 24969 decreased reward as measured by an increase in lateral hypothalamic ICSS reward thresholds; an effect blocked by pretreatment with the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (Harrison et al., 1999). RU 24969 may also attenuate increases in ICSS reward thresholds induced by cocaine. However, this has been attributed to the independent effects of RU 24969 and cocaine on ICSS thresholds canceling each other out rather than a blockade of cocaine's rewarding effect (Harrison et al., 1999). In agreement with 5-HT_{1B} receptor-mediated increases on ICSS thresholds, 5-

HT_{1B} receptor agonists also decrease operant responding for AMPH (Fletcher and Korth, 1999; Fletcher et al., 2002) and for ethanol self-administration (Maurel et al., 1999; Silvestre et al., 1998; Tomkins and O'Neill, 2000). In contrast to effects on AMPH and ethanol self-administration, 5-HT_{1B} receptor activation appears to potentiate the rewarding effects of cocaine in this paradigm (Parsons et al., 1998). The selective 5-HT_{1B} receptor agonist CP 94253 may also induce a dose-dependent CPA that is sensitive to blockade by GR 127935 (GR 127935 did not induce place conditioning on its own) (Cervo et al., 2002).

Although CP 94253 induced aversive place conditioning effects when administered alone, co-administration with a sub-threshold dose of cocaine induced positive reinforcing effects as measured by a CPP (Cervo et al., 2002). Pretreatment with GR 127935 blocked the acquisition of the CPP induced by CP 94253 plus cocaine (Cervo et al., 2002). With the exception of these studies on cocaine reinforcement, stimulation of 5-HT_{1B} receptors appears to induce aversive reinforcing effects.

It is well established that systemic administration of RU 24969 increases locomotor activity (Carli et al., 1988; Chaouloff et al., 1999; Cheetham and Heal, 1993; Goodwin and Green, 1985; Kalkman, 1995; Oberlander et al., 1987; Rempel et al., 1993; Tricklebank et al., 1986); intra-VTA, intra-subthalamic nucleus and intra-substantia nigra administration of RU 24969 (Martinez-Price and Geyer, 2002; Oberlander, 1983) also induced locomotor stimulant effects. Although many studies confirm that RU 24969 induces locomotor hyperactivity, there is debate as to which 5-HT receptor subtype(s) is responsible for this effect. There is evidence that RU 24969-induced hyperactivity may be mediated by stimulation of the 5-HT_{1A} receptor (Kalkman, 1995), the 5-HT_{1B} receptor

(Chaouloff et al., 1999; Cheetham and Heal, 1993; Martinez-Price and Geyer, 2002; Rempel et al., 1993) or via activation of both the 5-HT_{1A} and 5-HT_{1B} receptor subtypes (Martinez-Price and Geyer, 2002; O'Neill and Parameswaran, 1997). 5-HT_2 receptor blockade may also potentiate RU 24969 induced hyperactivity in rats (Goodwin and Green, 1985), although this result has not been replicated in mice (Cheetham and Heal, 1993; Goodwin and Green, 1985). The lack of agreement on 5-HT receptor subtype mediation of RU 24969-induced hyperactivity may be due to differences in the species tested (i.e. rat or mouse), procedural or locomotor activity measurements between laboratories or the selectivity of the antagonists used to investigate the 5-HT receptor subtypes of interest.

In this context, few studies have examined the effect of selective 5-HT_{1B} receptor agonists on locomotor activity. Systemic administration of the 5-HT_{1B} receptor agonist CP 94253 (with a 46-fold selectivity over the 5-HT_{1A} receptor) did not alter basal locomotor activity [except at a very high dose 32.0 mg/kg] (Koe et al., 1992; Lin and Parsons, 2002). Similarly, intra-VTA or intra-substantia nigra administration of the 5-HT_{1B} receptor agonist CP 93129 did not alter basal locomotor activity (Martinez-Price and Geyer, 2002; Papla et al., 2002); however, intra-subthalamic nucleus infusion of CP 93129 induced an increase in locomotor activity (Martinez-Price and Geyer, 2002).

The purpose of the present study was to examine and compare the effects of the selective 5-HT_{1B} receptor agonist CP 94253 and of the mixed 5-HT receptor agonist RU 24969 on place conditioning and locomotor activity. Based on a previous report (Cervo et al., 2002), it was hypothesized that CP 94253 would induce a CPA. As RU 24969 may decrease rewarding effects in other paradigms (e.g. ICSS) and is known to have a high

affinity for the 5-HT_{1B} receptor, it was hypothesized that RU 24969 may also induce a CPA. In addition, the current study also investigated the systemic effect of CP 94253 on locomotor activity and attempted to clarify which 5-HT receptor subtype was responsible for RU 24969-induced hyperactivity. It was hypothesized that systemic administration of CP 94253 may not alter locomotor activity (Koe et al., 1992); activation of both 5-$HT_{1A/B}$ receptors may contribute to RU 24969- induced locomotor hyperactivity (Martinez-Price and Geyer, 2002; O'Neill and Parameswaran, 1997). Effects of pretreatment with the selective 5-HT_{2C} receptor antagonist SB 242084 on RU 24969-induced hyperactivity was also examined as the non-selective 5-$HT_{2A/C}$ receptor antagonist ritanserin has been reported to attenuate RU 24969-induced hyperactivity in rats (Goodwin and Green, 1985).

Materials and Methods

Animals

Male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-250 g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights from 0700 h -1900 h) with food and water freely available. The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Place Conditioning

Apparatus: The place conditioning apparatus (I. Halvorson System Design, Phoenix, AZ, USA) consisted of a rectangular Plexiglas box divided into two compartments (30 cm L x 30 cm W x 25 cm H). The compartments differed only in floor texture: 14 horizontal bars positioned 1.25 cm apart compared with 1-cm square grate wire flooring. The compartments were separated by a white plastic divider, which contained a tunnel (7.5 cm long) allowing access to both compartments that could be obstructed with removable doors during conditioning.

Procedure: The procedure consisted of three phases. *Phase 1* (Pre-Conditioning): Animals (n= 8-16) were habituated to the place conditioning apparatus for three consecutive days, during which animals had free access to both compartments for 15 min. On the third day of pre-conditioning, the amount of time spent in each compartment was recorded to the nearest second. Independent experiments were conducted according to an unbiased and a biased place conditioning design. For the unbiased design, the animals were randomly assigned to each drug group and adjusted if necessary to equalize baseline compartment preference between and within each group.

In the biased design, because the induction of a CPA was expected following administration of RU 24969, animals were randomly assigned to drug groups such that they were each conditioned to the most preferred compartment, as determined on pre-conditioning day three. In the biased design the (+)-AMPH sulphate positive control group was conditioned to the least preferred side, as determined on pre-conditioning day three. This was arranged because the induction of a CPP was expected in this group.

Phase 2 (Conditioning): On alternating days, animals received drug and vehicle

treatment and were confined to the drug-paired or vehicle-paired compartment for 30 min. Animals received three drug- and three vehicle- paired conditioning sessions. *Phase 3* (Post-Conditioning): During retention testing, animals were placed in the apparatus in a drug-free state and allowed free access to both compartments for 15 min. The amount of time spent in each compartment was recorded to the nearest second. All testing took place under red light during the light phase of the light/dark cycle (lights on from 0700-1900 h). The place conditioning apparatus was cleaned between each animal with a diluted (1:6) ammonia-based window cleaner (No Name ® Glass Cleaner with ammonia).

Spontaneous Locomotor Activity

Apparatus: Spontaneous locomotor activity was measured using computer–monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA). The locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity as well as consecutive beam breaks. Vertical (rearing) activity was measured using 12 additional photobeams located 12 cm above the floor.

Procedure: Animals (n=7-8) were habituated to the locomotor activity boxes for two consecutive days (60 min/day) to establish baseline exploratory activity. Animals then received randomized counterbalanced injections with three drug-free days between injections. Locomotor activity was monitored for 60 min to explore the time course of the drug effects. All testing took place under red light during the light phase of the light/dark cycle. The photobeam boxes were cleaned between animals with an ammonia-

based window cleaner (No Name ® Glass Cleaner with ammonia) diluted with water (1:6).

Drugs

The mixed 5-HT receptor (5-$HT_{1A/B/2C}$) agonist RU 24969 · hemisuccinate [5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole hemisuccinate], the 5-HT_{1B} receptor agonist CP 94253 · HCl [5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine hydrochloride], and the 5-$HT_{1B/D}$ receptor antagonist GR 127935 · HCl [N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). The 5-HT_{2C} receptor antagonist SB 242084 · 2HCl [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride] and the 5-HT_{1A} receptor antagonist WAY 100635 maleate [N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl-cyclohexanecarboxamine maleate] were purchased from Sigma Chemical Company (St. Louis, MO, USA). (+)-AMPH sulphate was purchased from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). CP 94253, RU 24969, GR 127935, and SB 242084 were dissolved in doubled distilled water. AMPH and WAY 100635 were dissolved in 0.9% saline. SB 242084 was injected i.p. and all other drugs were injected s.c. All drugs were injected in a volume of 1.0 ml/kg and drug doses are expressed as free-base.

Drug Treatment

Experiment 1: The effects of systemic RU 24969 or CP 94253 on place conditioning

The effects of the mixed 5-HT receptor agonist RU 24969 (1.0 - 5.0 mg/kg, s.c., 10 min prior) on place conditioning were examined. Animals received three injections of RU 24969 or vehicle on alternating days. The effects of RU24969 were tested in both an unbiased and biased place conditioning design [Experiment 1a]. The reinforcing effects of RU 24969 were initially examined using an unbiased place conditioning design. However, due to the lack of place conditioning effects using this procedure (see results), the effect of RU 24969 on place conditioning was further investigated using a biased place conditioning design. A biased place conditioning design was used as it may maximize the chance of observing conditioning effects (Bozarth, 1987; Cunningham et al., 2003).

The effects of the selective 5-HT_{1B} receptor agonist CP 94253 (1.25 and 2.5 mg/kg, s.c., 10 min prior) on place conditioning were examined using an unbiased place conditioning design. Animals received three injections of CP 94253 or vehicle on alternating days [Experiment 1b]. As place conditioning effects were observed following CP 94253 using an unbiased place conditioning design (see results), the effects of CP 94253 in a biased place conditioning design were not examined In all place conditioning experiments, a positive control group which received (+)-AMPH (1.0 mg/kg, s.c., 10 min prior) was included.

Experiment 2: Effects of 5-HT receptor antagonists on RU 24969- induced hyperactivity

Separate groups of animals received randomized counterbalanced injections as follows: RU 24969 (2.5 mg/kg, s.c., 10 min prior) and the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg, s.c., 15 min prior) alone and in combination and with vehicle [Experiment 2a]; RU 24969 (2.5 mg/kg, s.c., 10 min prior) and the 5-HT_{1B} receptor antagonist GR 127935 (3.0 mg/kg, s.c., 30 min prior) alone and in combination and with vehicle; RU 24969 (2.5 mg/kg, s.c., 10 min prior) and the 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg, i.p., 30 min prior) alone, in combination and with vehicle [Experiment 2b]. Locomotor activity was measured over a 60 min period.

Experiment 3: Effect of systemic CP 94253 on locomotor activity

Animals received a randomized, counterbalanced series of four injections; vehicle or CP 94253 (2.5 – 10.0 mg/kg, s.c., 10 min prior to testing) and locomotor activity was measured over a 60 min period.

Statistical Analysis

Experimental effects on place conditioning were determined by paired sample t-tests to compare time spent in the drug-paired side on pre-conditioning day 3 and post-conditioning day 1 ($P \leq 0.05$). Experimental effects on locomotor activity were determined using repeated measures ANOVA. CP 94253 dose-response data was analyzed using two-way repeated measures ANOVA (drug x time). Drug interaction data for experiments involving RU 24969 were analyzed a using three-way repeated measures ANOVA [RU 24969 x antagonist x time]. Experimental effects of GR 127935 and SB 242084 on RU 24969-induced hyperactivity were analyzed together treating antagonist as

a factor with two levels. This approach was used as effects of these drugs were tested in the same group of animals. Where appropriate, analysis of time course data using one-way repeated measures ANOVA across all drug treatment groups at each 5 min interval was conducted. A significant F ratio ($P \leq 0.05$) on a 5 min interval was followed by comparison of each drug condition to vehicle using Newman-Keul's post hoc tests (α=0.05). Only the results on horizontal locomotor activity are reported. All statistical analyses were completed using SPSS statistical software (SPSS 13.0, SPSS Inc., Chicago, U.S.A.).

Results

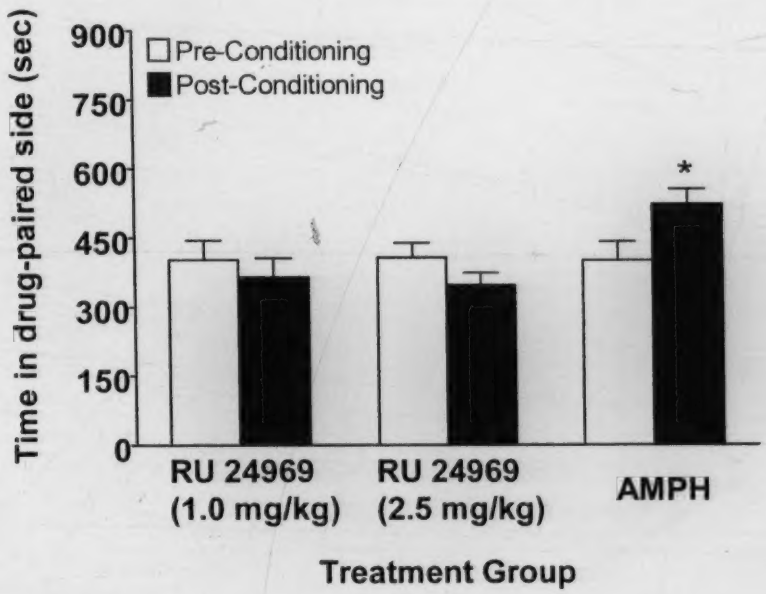
Experiment 1: The effect of systemic RU 24969 or CP 94253 on place conditioning

Experiment 1a: RU 24969 (1.0, 2.5 or 5.0 mg/kg) did not induce place conditioning when tested in an unbiased place conditioning design [1.0 mg/kg - $t(11) = 0.660$; 2.5 mg/kg - $t(11) = 2.047$; 5.0 mg/kg - $t(7) = 0.849$, $P > 0.05$]. (+)-AMPH induced a CPP [$t(11) = 2.53$, $P<0.05$; $t(7) = 4.456$, $P <0.05$] in each of these experiments (Figure 4.1A and Figure 4.1B).

In a biased place conditioning design, RU 24969 (2.5 mg/kg) did not induce place conditioning effects [$t(7) = 2.131$, $P>0.05$] (Figure 4.2A); however, RU 24969 (5.0 mg/kg) did induce a significant CPA [$t(15) = 3.197$, $P>0.05$] (Figure 4.2B). (+)-AMPH induced a significant CPP [$t(7) = 6.278$; $t(7) = 4.367$, $P <0.05$] in both experiments.

Figure 4.1. Effects of RU 24969 (1.25 and 2.5 mg/kg) and (+)-AMPH (1.0 mg/kg) (A) and RU 24969 (5.0 mg/kg) and (+)-AMPH (1.0 mg/kg) (B) in an unbiased place conditioning procedure. Data shown are means ± S.E.M. * Significant $P<0.05$.

A



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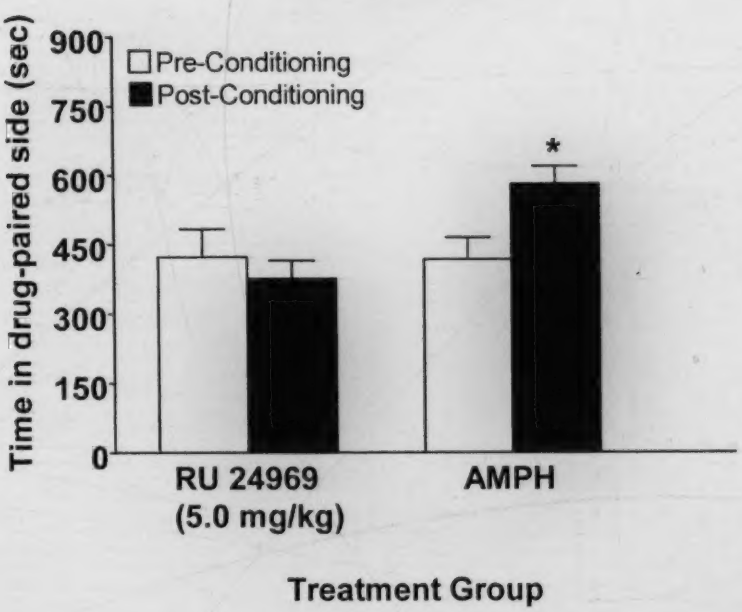
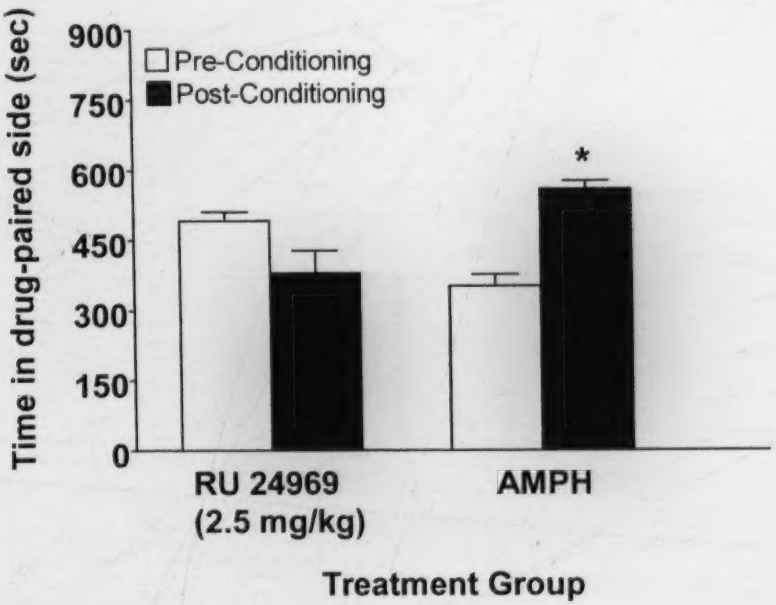


Figure 4.2. Effects of RU 24969 (2.5 mg/kg) and (+)-AMPH (1.0 mg/kg) (A) and RU 24969 (5.0 mg/kg) and (+)-AMPH (1.0 mg/kg) (B) in a biased place conditioning procedure. Data shown are means ± S.E.M. * Significant $P<0.05$.

A



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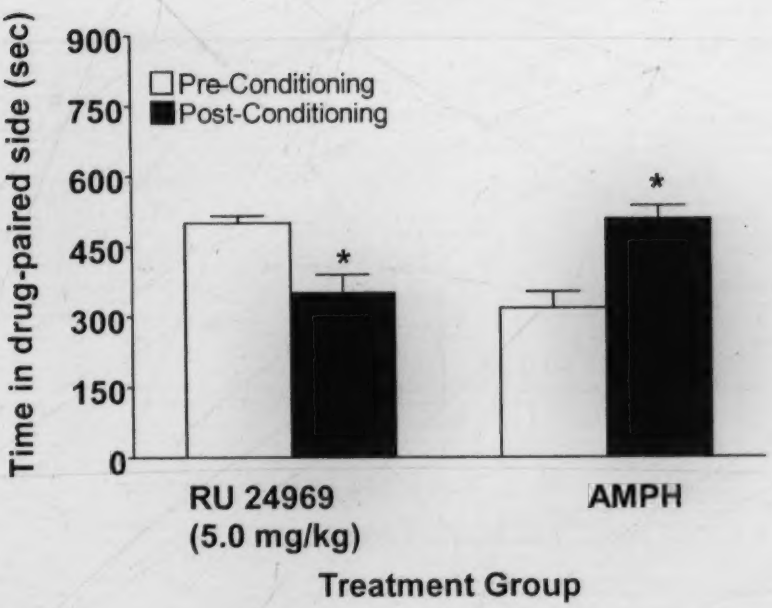
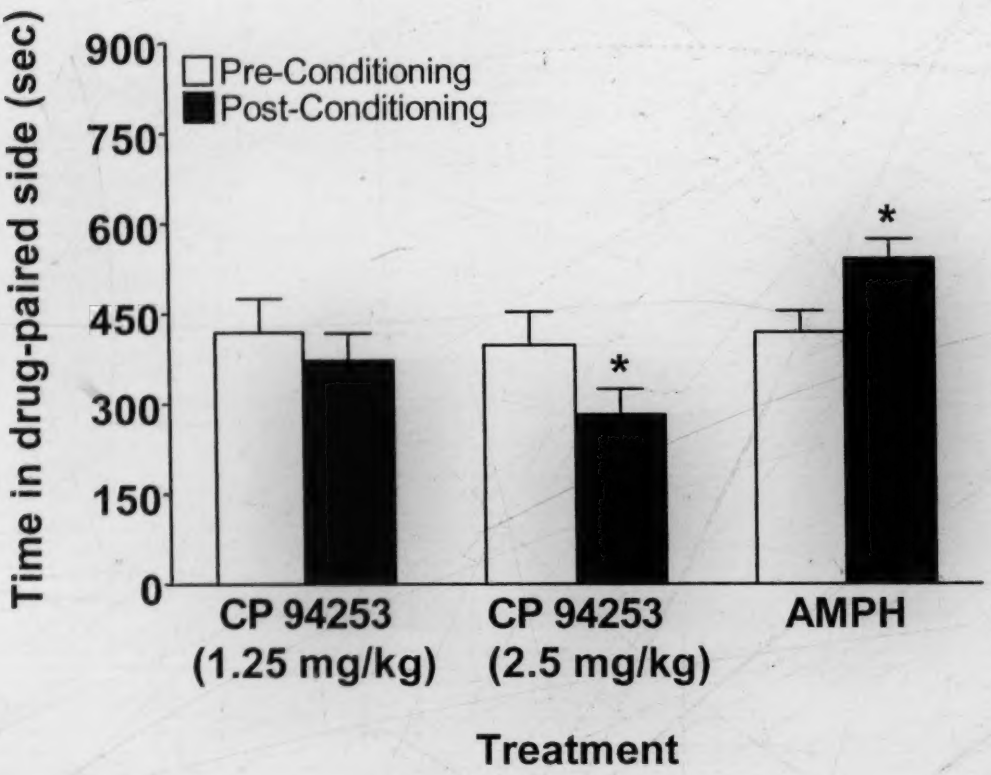


Experiment 1b: The 5-HT_{1B} receptor agonist CP 94253 (2.5 mg/kg) induced a CPA in an unbiased place conditioning design [$t(7) = 2.526$, $P < 0.05$]; CP 94253 (1.25 mg/kg) did not induce place conditioning [$t(7) = 0.769$; $P>0.05$]. Under these conditions (+)-AMPH induced a significant CPP [$t(7) = 2.664$, $P < 0.05$] (Figure 4.3).

Figure 4.3. Effects of CP 94253 (1.25 and 2.5 mg/kg) and (+)-AMPH (1.0 mg/kg) in an unbiased place conditioning procedure. Data shown are means ± S.E.M. * Significant $P<0.05$.

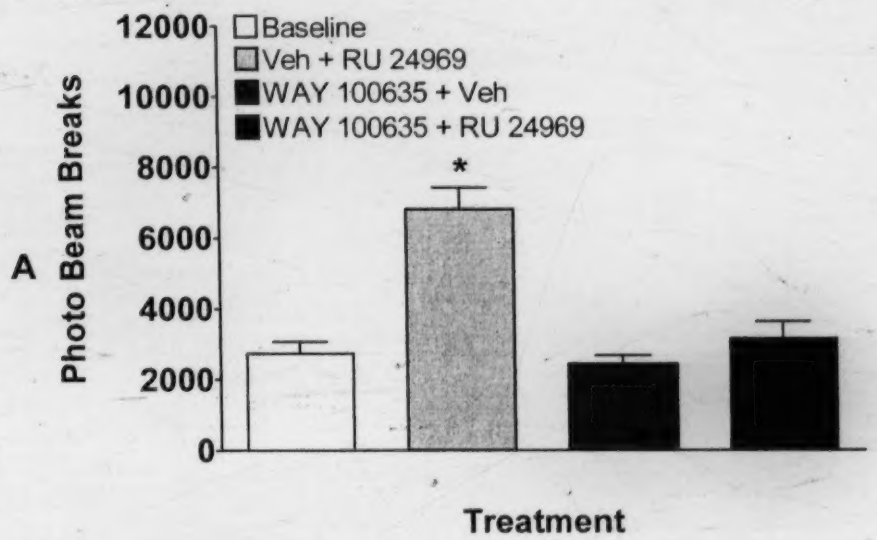


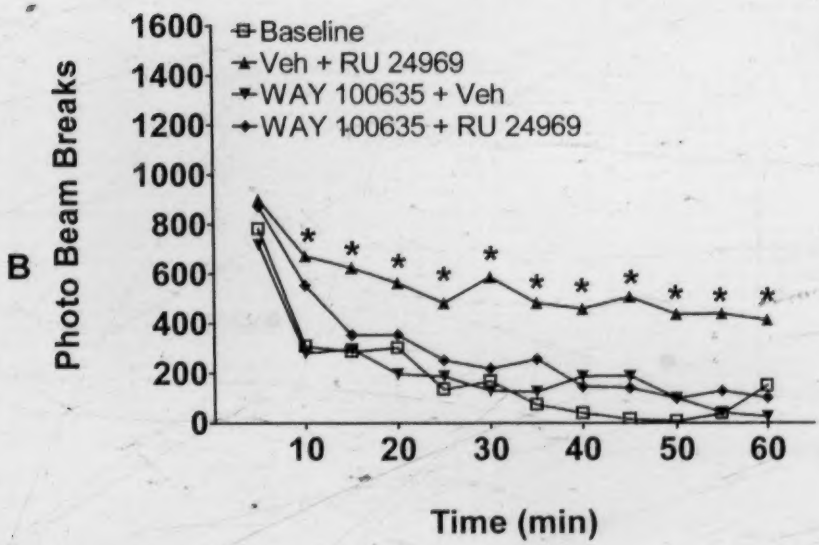
Experiment 2: Effects of 5-HT receptor antagonists on RU 24969- induced hyperactivity

Experiment 2a: RU 24969 increased locomotor activity [F (1, 6) = 71.094, P<0.05]. There was also a significant main effect of the 5-HT_{1A} receptor antagonist WAY 100635 [F (1, 6) = 20.288, P< 0.05], and time [F (2.312, 13.871) = 34.982, P<0.05]. Significant interactions of RU 24969 x WAY 100635 [F (1, 6) = 19,602, P<0.05] and RU 24969 x WAY 100635 x time [F (3.283, 19.697) = 3.009, P<0.05] were also observed (Figure 4.4A). The interaction of RU 24969 x WAY 100635 x time indicated that pretreatment with WAY 100635 blocked the locomotor increasing effect induced by RU 24969.

RU 24969 significantly increased locomotor activity compared to vehicle during the last 55 min of the 60 min test session. Pretreatment with WAY 100635 significantly blocked RU 24969-induced locomotor hyperactivity during the last 45 min of the test period (15-60 min time period). WAY 100635 + RU 24969 was significantly different from RU 24969 treatment alone from 15-60 min; however it was not significantly different than control treatment (Figure 4.4B).

Figure 4.4. Effects of RU 24969 (2.5 mg/kg) and the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.

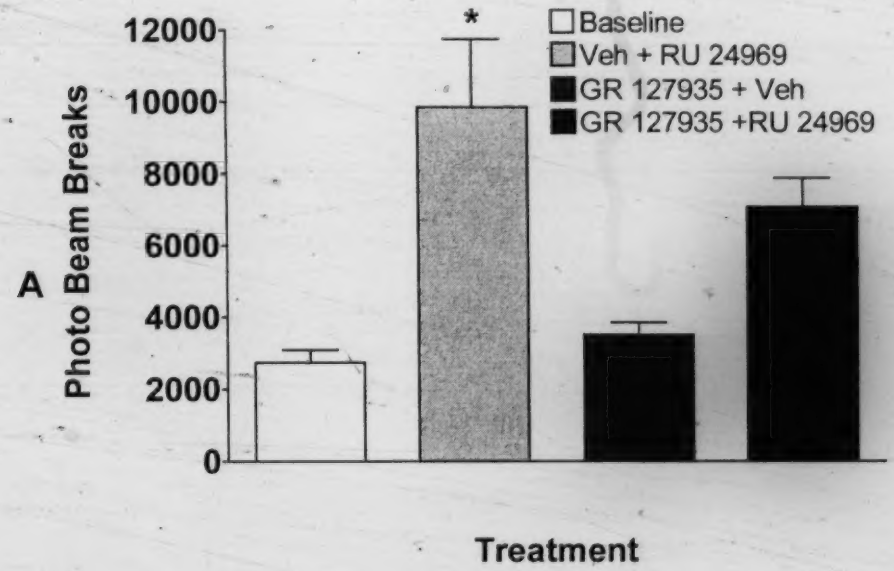




Experiment 2b: Three-way repeated measures ANOVA (RU 24969 x antagonist x time) revealed that there was a significant effect of RU 24969 [$F(1,7)=22.995$, $P<0.05$] and time [$F(1.868, 13.077)=48.502$, P, 0.05]. There were also significant interactions of RU 24969 x time [$F(3.495, 24.462)=3.376$, $P<0.05$] and RU 24969 x antagonist x time [$F(4.684, 32.789) =3.429$, $P<0.05$] (Figure 4.5A and Figure 4.6A).

Local time course analysis revealed that RU 24969 significantly increased locomotor activity compared to vehicle at the 20, 25 and 45-60 min time periods. Animals treated with GR 127935 + RU 24969 were not significantly different from either control or RU 24969 treatment alone throughout the 60 min test period (Figure 4.5B). The locomotor activity of animals treated with SB 242084 + RU 24969 was significantly greater than control during the 20-30 and 40-60 min test periods; however throughout testing, this treatment group was not significantly different than RU 24969 treatment alone (Figure 4.6B).

Figure 4.5. Effects of RU 24969 (2.5 mg/kg) and the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (3.0 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



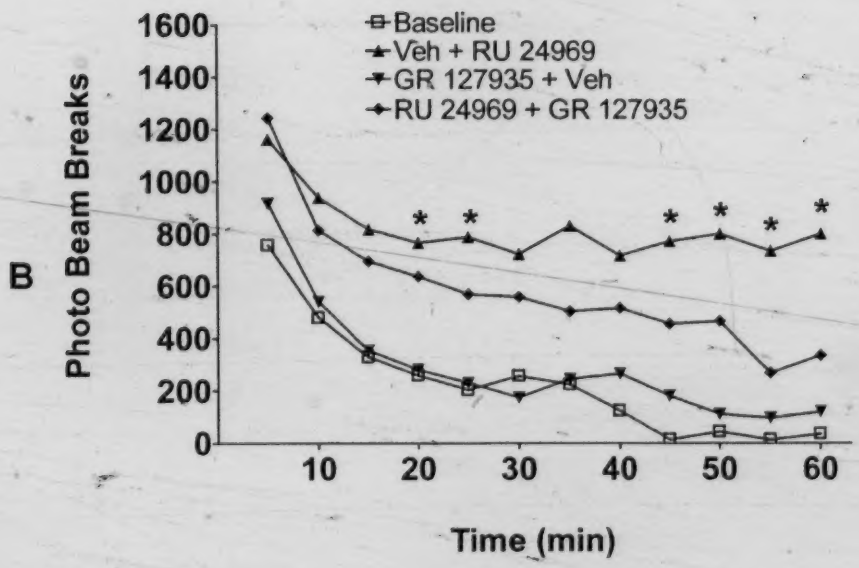
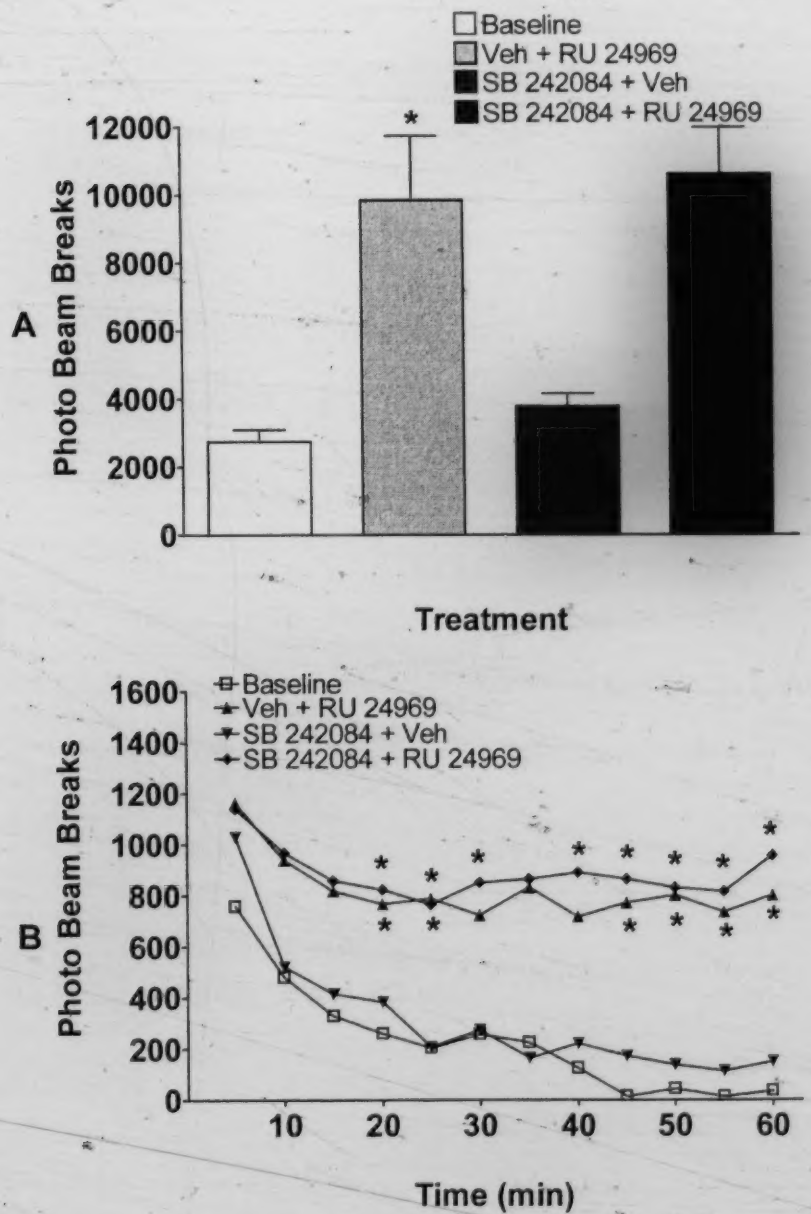


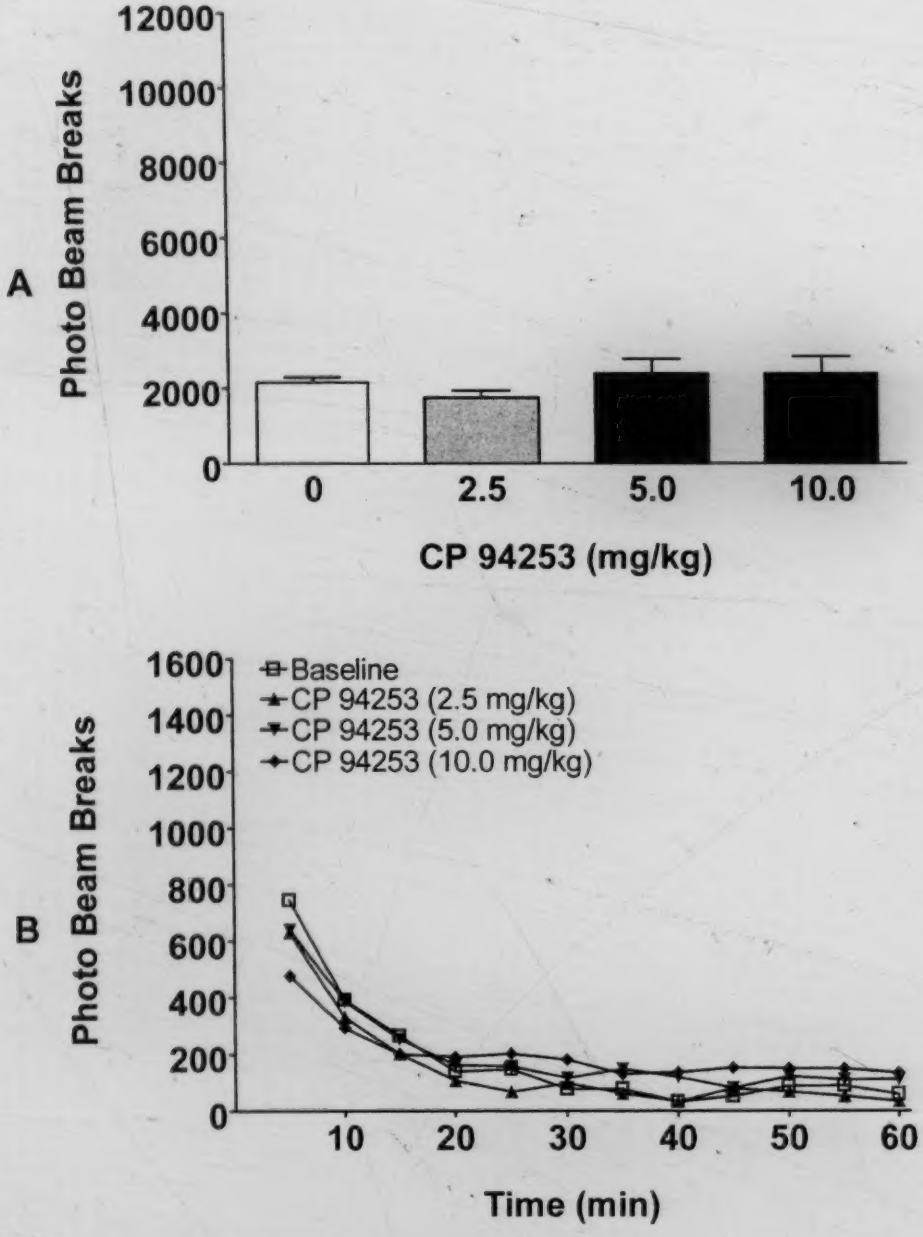
Figure 4.6. Effects of RU 24969 (2.5 mg/kg) and the 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 3: Effects of selective 5-HT_{1B} receptor agonist CP 94253 on locomotor activity

Two-way repeated measures ANOVA revealed that there was no effect of CP 94253 (2.5-10.0 mg/kg) on locomotor activity. There was a significant main effect of time [$F(3.571, 24.998) = 75.746$, $P<0.05$); however no CP 94253 x time interaction was observed (Figure 4.7A and Figure 4.7B).

Figure 4.7. Lack of effect of the 5-HT_{1B} receptor agonist CP 94253 (0-10.0 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity (B) over 60 min. Data shown are means ± S.E.M.



Discussion

In the present study, both the selective 5-HT_{1B} receptor agonist CP 94253 and the mixed 5-HT receptor (5-$HT_{1A/B/2C}$) agonist RU 24969 induced a CPA. The CPA observed by CP 94253 in the current report is consistent with that reported by Cervo et al. (2002). However, to our knowledge this is the first report of aversive reinforcing effects of RU 24969 in the place conditioning paradigm. Although activation of 5-HT_{1B} receptors increases mesocorticolimbic DA release (Iyer and Bradberry, 1996; O'Dell and Parsons, 2004; Yan and Yan, 2001b; Yan et al., 2004), an effect that may be associated with an increase in reward/reinforcement (Blaha and Phillips, 1990; Hernandez and Hoebel, 1988; Pettit and Justice, 1991; Pierce and Kumaresan, 2006; Tzschentke, 1998; Wise, 2004), stimulation of this receptor is consistently reported to induce reward-decreasing effects (Cervo et al., 2002; Fletcher and Korth, 1999; Fletcher et al., 2002; Harrison et al., 1999; Hayes et al., 2006; Tomkins and O'Neill, 2000); thus 5-HT_{1B} receptors may not alter place conditioning via modulation of mesocorticolimbic DA. In addition to altering DA neurotransmission, 5-HT_{1B} receptor stimulation also alters extracellular levels of other neurotransmitters such as 5-HT, GABA, glutamate and acetylcholine. For example, stimulation of 5-HT_{1B} receptors decreases GABA release (Johnson et al., 1992; O'Dell and Parsons, 2004; Parsons et al., 1999; Yan and Yan, 2001a; Yan et al., 2004). Blockade of $GABA_A$ receptors by systemic administration of picrotoxin induced a CPA (Acquas et al., 1990; File, 1986); it is possible that 5-HT_{1B} receptor stimulation induces aversive reinforcing effects by altering the release of other neurotransmitters such as GABA.

Although it is unknown why stimulation of 5-HT_{1B} receptors induces aversive reinforcing effects in the place conditioning test, previous reports have suggested that 5-HT_{1B} receptor agonists induce anxiogenic effects in behavioural tests (Benjamin et al., 1990; Lin and Parsons, 2002; Pellow et al., 1987); however, this result has not been consistently reported (Bell et al., 1995; Chojnacka-Wojcik et al., 2005). In addition, compounds which are known to be anxiogenic such as yohimbine, picrotoxin, and BIBP3226 (neuropeptide Y antagonist) induce CPAs (Acquas et al., 1990; File, 1986; Kask et al., 1999; Spyraki et al., 1985). As 5-HT_{1B} receptor stimulation may induce anxiogenic effects, and other compounds that are known to be anxiogenic induce CPA, it is possible that the CPA induced by CP 94253 or RU 24969 administration may be related to the anxiogenic effects of these compounds. Stimulation of 5-HT_{2C} receptors also induces anxiogenic effects (Alves et al., 2004; Kennett et al., 1989); however, activation of the 5-HT_{2C} receptor induced a state-dependent CPA (Mosher et al., 2006). It was hypothesized that the state-dependent nature of the CPA induced by 5-HT_{2C} receptor stimulation may be due to alterations in the animals' sensory perception as opposed to the induction of anxiety states (Mosher et al., 2006). Thus, 5-HT_{1B} and 5-HT_{2C} receptor activation may each induce conditioned aversive effects; however, they may do so by different mechanism (e.g. anxiety state vs. sensorimotor alterations).

In the current study, RU 24969 did not induce place conditioning effects when animals were tested using an unbiased place conditioning design. Therefore, the effects of RU 24969 on place conditioning were further examined using a biased place conditioning design. A biased place conditioning design was used as it may maximize the probability of observing conditioning reinforcing effects (Bozarth, 1987; Cunningham

et al., 2003). Administration of RU 24969 induced a CPA when animals were tested in a biased place conditioning design. Selective activation of 5-HT_{1B} receptors by CP 94253 also induced a CPA, but this effect was observed using an unbiased place conditioning design; therefore, place conditioning effects of CP 94253 were not investigated using a biased place conditioning design. This result is consistent with both RU 24969 (Harrison et al., 1999) and CP 94253 (Hayes et al., 2006) inducing reward-decreasing effects as measured by an increase in ICSS thresholds.

Previous reports of 5-HT_{1A} receptor activation using 8-OHDPAT have shown that in general, stimulation of this receptor induces a CPP in an unbiased place conditioning design (Fletcher et al., 1993; Papp and Willner, 1991; Shippenberg, 1991). In a biased place conditioning design, low doses of 8-OHDPAT induced a CPP; however, when a high dose of 8-OHDPAT (1.0 mg/kg) was tested, a CPA was observed (Papp and Willner, 1991). Studies on lateral hypothalamic ICSS have reported that intra-MRN administration of 8-OHDPAT may induce rewarding effects (Fletcher et al., 1995) whereas administration of RU 24969 may induce reward decreasing effects in this behavioural test (Harrison et al., 1999). In addition, 8-OHDPAT and RU 24969 induce differential changes on locomotor activity in nicotine- and saline- treated animals (Chapter 5). 8-OHDPAT may increase locomotor activity in saline- but not nicotine-treated animals; administration of RU 24969 may increase locomotor activity in animals treated with either saline or nicotine. These studies indicate that selective 5-HT_{1A} receptor activation by 8-OHDPAT or administration of RU 24969 may have differential effects on behaviour. These results indicate that the reinforcing effects induced by RU 24969 may be mediated by activation of the 5-HT_{1B} receptor or stimulation of multiple 5-

HT receptor subtypes. Further studies on place conditioning should be undertaken using a wider dose range of RU 24969 and selective antagonists for the 5-HT_{1A} and 5-HT_{1B} receptors.

In the current study, the mixed 5-HT receptor agonist RU 24969 induced locomotor hyperactivity; an effect that was reversed (i.e. same as control levels) by antagonism of the 5-HT_{1A} receptor and decreased by 5-HT_{1B} receptor blockade. Although pretreatment with the 5-$HT_{1B/D}$ receptor antagonist GR 127935 did not completely reverse the locomotor increasing effects of RU 24969, there was a significant effect of GR 127935 on RU 24969-induced hyperactivity as indicated by the significant RU 24969 x antagonist x time [$F(4.684, 32.789) = 3.429$, $P<0.05$] interaction. The results of the present study indicate that the 5-HT_{1A} and possibly the 5-HT_{1B} receptor subtypes may mediate the effects of RU 24969 on locomotor activity; however the 5-HT_{1A} receptor may be more involved in this process than the 5-HT_{1B} receptor. The result of 5-HT_{1A} receptor antagonism reversing RU 24969-induced locomotor hyperactivity to control levels observed in the current study are in agreement with the findings of Kalkman et al. (1995). Pretreatment with the 5-$HT_{1B/D}$ receptor antagonist GR 127935 also decreased the locomotor stimulant effect of RU 24969; the combination of GR 127935 + RU 24969 was not significantly different from control. This result is consistent with that reported by O'Neill and Parameswaran (1997) indicating that activation of the 5-HT_{1B} receptor may contribute in part to the locomotor hyperactivity induced by RU 24969. In contrast, Kalkman et al. (1995) reported that GR 127935 did not alter the locomotor stimulant effect of RU 24969. However, a lower dose of GR 127935 (1.0 mg/kg) was used in that study compared to that used in the current study (3.0 mg/kg) and

that of O'Neill and Parameswaran (1997) (1.0-5.0 mg/kg) and Chaouloff et al. (1999) (1.0-10.0 mg/kg). In addition, O'Neill and Parameswaran (1997), Chaouloff et al. (1999) and the present study measured the effects of RU 24969 on locomotor activity for a 60-90 min period compared to 15 min by Kalkman et al. (1995).

Blockade of the 5-HT_{2C} receptor by SB 242084 did not alter RU 24969-induced hyperactivity in the current study. The 5-$HT_{2A/C}$ receptor antagonist ritanserin potentiated the locomotor stimulant effects of RU 24969 in rats(Goodwin and Green, 1985), however the present results indicate that effect may be due to blockade of the 5-HT_{2A} receptor. The lack of 5-HT_{2C} receptor antagonist effects in the current report is consistent with studies in mice as the 5-$HT_{2A/C}$ receptor antagonists ketanserin and ritanersin did not alter RU 24969- induced locomotor hyperactivity in that context (Cheetham and Heal, 1993; Goodwin and Green, 1985).

Intra-VTA, but not intra-NAS, administration of RU 24969 may induce locomotor hyperactivity (Oberlander, 1983). In addition, systemic or intra-VTA administration of RU 24969 potentiated the locomotor stimulant effect of intra-NAS or systemic administration of AMPH respectively (Oberlander, 1983). Increases in mesocorticolimbic DA has been associated with an increase in locomotor activity (Benwell et al., 1995; Di Chiara and Imperato, 1988; Dunnett and Robbins, 1992; Mathe et al., 1996). Activation of 5-HT_{1B} heteroreceptors reduces GABA inhibition of mesocorticolimbic DA neurons, thus leading to an increase in DA transmission (Guan and McBride, 1989; Johnson et al., 1992; Parsons et al., 1999; Yan and Yan, 2001a; Yan et al., 2004). Therefore, the locomotor stimulant effects induced by RU 24969 may be partially mediated via stimulation of 5-HT_{1B} receptor on VTA GABA neurons. Intra-

MRN administration of the 5-HT_{1A} receptor agonist 8-OHDPAT may increase locomotor activity (Ahn et al., 2005; Higgins and Elliott, 1991). Neurons of the MRN project to the mesocorticolimbic system (Azmitia and Segal, 1978; Herve et al., 1987), and it is possible that systemically administered RU 24969 stimulates 5-HT somatodendritic autoreceptors in the MRN, resulting in decreased 5-HT inhibitory effects on DA-containing neurons and leading to an increase in locomotor activity. This hypothesis is consistent with the observation that DA depletion following reserpine treatment may reduce RU 24969-induced locomotor hyperactivity in mice (Cheetham and Heal, 1993).

In accord with previous reports (Koe et al., 1992; Lin and Parsons, 2002), systemic administration of the selective 5-HT_{1B} receptor agonist CP 94253 did not alter locomotor activity. As the effects of RU 24969 are not consistent with that of selective 5-HT_{1B} receptor stimulation on locomotor activity, this may further indicate that the hyperactivity induced by RU 24969 may not be fully mediated via the 5-HT_{1B} receptor.

Although RU 24969 is generally cited as a 5-HT_{1B} receptor agonist, the current experiment demonstrates that the locomotor stimulant effect of RU 24969 in rats may be mediated by activation of 5-HT_{1A} receptors (Kalkman, 1995). The aversive reinforcing effects induced by CP 94253 and RU 24969 in the place conditioning test may be due to the induction of anxiety states or reward-decreasing effects following systemic administration of these compounds. In general, the results of the current study are consistent with previous reports of 5-HT_{1B} receptor stimulation and RU 24969 administration reducing reward and reinforcement. In addition, behaviours induced by RU 24969 may be due to activation of multiple 5-HT receptor subtypes such as the 5-HT_{1A} and 5-HT_{1B} (Martinez-Price and Geyer, 2002; O'Neill and Parameswaran, 1997).

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Chapter 5. 5-HT receptor mediated effects on nicotine-induced locomotor hyperactivity in rats

(Experiments with WAY 161503 and CP 94253 were conducted by T Mosher; all other experiments were conducted by S Waddock and have been reported in thesis form (Waddock, 1997). The Waddock data have been reanalyzed and presented here because they form part of the paper to be submitted. The representation and discussion of the Waddock data for this paper have been prepared by the present author with full acknowledgment of the source of those data. A version of this chapter will be submitted for publication. Mosher TM, Waddock SL and Greenshaw A 2006. 5-HT receptor mediated effects on nicotine-induced locomotor hyperactivity in rats. Neuropharmacology).

Introduction:

Repeated administration of nicotine may induce locomotor hyperactivity in rats (Morrison and Stephenson, 1972; Stolerman et al., 1973), with the (-)-nicotine enantiomer being ten times more potent than the (+)-nicotine enantiomer in this context (Clarke and Kumar, 1983b); effects blocked by administration of the nAch receptor antagonist mecamylamine (Benwell et al., 1995; Clarke and Kumar, 1983a, b). The role of central nAch receptors in this context is indicated by the inactivity of systemic injections of the peripherally acting nAch receptor antagonists chlorisondamine or hexamethadone (Benwell et al., 1995; Clarke and Kumar, 1983a, b), in contrast to the blockade of nicotine-induced hyperactivity by intracerebroventricular chlorisodamine (Clarke and Kumar, 1983b). Locomotor stimulant effects of the nAch receptor agonist

cytisine injected into the VTA also supports a central mechanism (Museo and Wise, 1990, 1994, 1995; Reavill and Stolerman, 1990).

Nicotine-induced hyperactivity in rats is normally mediated by activation of the mesocorticolimbic DA system. Systemic administration of nicotine increases extracellular DA concentrations in the NAS (Benwell and Balfour, 1992; Benwell et al., 1995; Damsma et al., 1989; Imperato et al., 1986; Nisell et al., 1994b; Nisell et al., 1996; Olausson et al., 2001), the VTA (Rahman et al., 2003) and the medial PFC (Clarke et al., 1988; Nisell et al., 1996). In addition, administration of nicotine into the VTA or NAS may increase extracellular DA concentrations within the NAS (Nisell et al., 1994a). Nicotine-induced increases in NAS DA release may be blocked by intra-VTA, but not intra-NAS, injection of mecamylamine (Nisell et al., 1994b). Systemic administration of nicotine increases the burst firing of DA neurons within the VTA (Mereu et al., 1987; Nisell et al., 1996) and the substantia nigra pars compacta, however, this effect was more pronounced within the VTA (Mereu et al., 1987) and is blocked by mecamylamine (Mereu et al., 1987).

Bilateral infusion of the neurotoxin 6-hydroxydopamine into the NAS abolished the increase in locomotor activity following nicotine administration as well as decreased DA concentrations in the NAS and olfactory tubercule (Clarke et al., 1988). In contrast, nicotine-induced hyperactivity was still observed in rats treated neonatally with intra-VTA 6-hydroxydopamine (Vezina et al., 1994). This discrepancy is attributable to developmental neuroplasticity and does not detract from the relationship of effects of repeated nicotine administration on mesocorticolimbic DA effects in the intact adult rat.

Within the mesocorticolimbic system, 5-HT and DA are known to have functional interactions. As the mesocorticolimbic DA system is involved in nicotine-induced hyperactivity, it was of interest to assess the effects of 5-HT receptor-related compounds on nicotine-induced hyperactivity. Experiments in the current report examined this hypothesis by testing compounds that act selectively at the 5-HT_{1A} receptor (8-OHDPAT), the 5-HT_{1B} receptor (CP 94253) and the 5-HT_{2C} receptor (WAY 161503). The less selective 5-HT receptor-related compounds NAN 190, RU 24969 and TFMPP were also tested in an attempt to provide a functional response profile. It was hypothesized that stimulation of autoreceptors (i.e. 5-HT_{1A} and 5-HT_{1B} receptors) would enhance both spontaneous and nicotine-induced hyperactivity whereas these behaviours may be attenuated by activation of post-synaptic 5-HT_{2C} receptors.

Materials and Methods

Animals

Male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-250 g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights from 0700 h -1900 h) with food and water freely available. The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Spontaneous Locomotor Activity

Apparatus: Spontaneous locomotor activity was measured using computer-monitored photobeam boxes (I. Halvorson System Design, Phoenix, AZ, USA). The locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity as well as consecutive beam breaks. Vertical activity (rearing) was measured using 12 additional photobeams located 12 cm above the floor.

Procedure: Animals were habituated to the locomotor activity boxes for 14 days to establish baseline exploratory activity. During these 14 days, animals were injected daily with either (-)-nicotine (0.6 mg/kg, s.c.) or saline, depending on the treatment group to which they were randomly assigned. The animals were injected daily in order to establish behavioural sensitization to nicotine. Following the 14 day sensitization period, the animals received randomized counterbalanced injections with the compound of interest; there were three days between each drug-probe injection. During the three days between probe injections, the animals were injected with either saline or nicotine and tested in the locomotor activity boxes. Locomotor activity was monitored for 30 or 60 min/day under red light conditions during the light phase of the light/dark cycle. WAY 161503 and CP 94253 were tested over 60 min as few studies have examined the effects of these compounds on locomotor activity; the remainder of the drugs were tested over a 30 min period as they have previously been reported to induce locomotor effects during that time frame. The photobeam boxes were cleaned between each animal with an ammonia-based window cleaner (No Name ® Glass Cleaner with ammonia) diluted with water (1:6).

Drugs

The 5-HT_{1A} receptor agonist 8-OHDPAT · HBr [(±)-8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide], the partial 5-HT_{1A} receptor agonist NAN 190 · HBr [1-(2- methoxyphenyl)-4-[4-(2-pthalimido) butyl] piperazine hydrobromide], the 5-$HT_{1A/B}$ receptor antagonist (±)-pindolol, the mixed 5-HT (5-$HT_{1A/B/2C}$) receptor agonist TFMPP · HCl [N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride], the 5-HT_{2C} receptor antagonist SB 242084 · 2HCl [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride] and (-)- nicotine hydrogen tartrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 5-HT_{1A} receptor antagonist WAY 100635 [N-(2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl)-N-(2-pyridinyl) cyclohexane carboxamine trihydrochloride] was purchased from Wyeth Ayerst. The mixed 5-HT (5-$HT_{1A/B/2C}$) receptor agonist RU 24969 [5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole was purchased from Rossel-Uclaf (Paris, Fr.). The 5-HT_{2C} receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride] and the 5-HT_{1B} receptor agonist CP 94253 · HCl [5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo[3,3-b]pyridine hydrochloride] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). All drugs were dissolved in either double distilled water or 0.9% saline. SB 242084 was injected i.p. and all other drugs were injected s.c. in a volume of 1.0 ml/kg (with the exception of intra-VTA microinjections described below). All drug doses are expressed as free-base.

Stereotaxic Surgery (Experiment 4a&b)

Following the 14-day sensitization period with either saline or (-)-nicotine, the animals were anaesthetized using halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA) and placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). The animals were unilaterally implanted on the right side of the VTA with a stainless steel 22-gauge guide cannula (Plastics One Inc., Roanoke, VA, USA). To avoid damage to the blood sinuses and the cerebral ventricles, the cannula was implanted using angular coordinates (Greenshaw, 1997). The stereotaxic coordinates used were interpolated from the target site and were defined using the rat brain atlas by Paxinos and Watson (1986). The coordinates are relative to interaural zero with the incisor bar 2.4 mm below the interaural line: VTA AP +3.2 mm, LM +0.6 mm, DV 2.3 mm, 20° lateral and anterior to the sagittal and coronal planes respectively. The tip of the guide cannula was placed 1.0 mm above the target site and were attached to the skull using dental acrylic (Caulk/Dentsply, Milford, DE, USA) and stainless steel screws (3.6 mm long and 0.8 mm in diameter; Lomat Watch Co., Montréal, QC, CA). Dummy cannulae (Plastics One Inc.) were left in place between microinjection procedures to prevent occlusion. Behavioural testing began at least one week following surgery; however, animals continued to receive daily injections of either saline or nicotine during the recovery period.

Microinjection Procedure (Experiments 4a&b)

During the microinjection procedure, the dummy cannula was removed and a 28-gauge injection cannula (Plastics One Inc.) was slowly lowered into the guide cannula.

The tip of the injection cannula extended 1.0 mm beyond the tip of the guide cannula into the target site. Microinjections (0.5 μL at 0.2 μL/min) were delivered over 2.5 min using 0.03 mg/m Accu-rated pump tubes (Fisher Scientific Ltd., Nepean, ON, CAN) and a 10-μL glass microsyringe (Hewlett-Packard, Mississauga, ON, CAN) attached to a Bee Hive Controller (Bioanalytical Systems Inc., West Lafayette, IN, USA). Following infusion of the drug, the injection cannula remained in place for one min to allow for drug absorption and diffusion. Behavioural testing took place immediately following the microinjection procedure.

Histology

Upon completion of each microinjection experiment, animals were anaesthetized using sodium pentobarbital (Euthanyl) (Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) and perfused intracardially with ice cold isotonic saline followed by 10% w/v buffered formalin phosphate (Fisher Scientific Ltd., Nepean, ON, Canada). Once brains were removed, they were placed in 10% w/v buffered formalin phosphate for 4-6 hr, before being placed in 30 % sucrose/10mM PBS buffer for 24 hr before freezing. Cannula placements were verified by inspection of 30 μm coronal brain slices. Only animals with correct cannula placements were included in the statistical analysis.

Experimental Designs

Two groups of animals were used for each experiment described below. One group of animals was chronically injected with saline (s.c.) to examine spontaneous

locomotor activity and one group was injected chronically with (-)-nicotine (0.6 mg/kg, s.c.).

Experiments 1a&b: Effect of systemic 5-HT$_{1A}$ receptor ligand administration on spontaneous and nicotine-induced locomotor activity

Groups of animals received randomized, counterbalanced series of four injections of either: vehicle or the 5-HT$_{1A}$ receptor agonist 8-OHDPAT (0.003, 0.03 or 0.3 mg/kg, s.c., 15 min prior to testing) [Experiment 1a]; vehicle or the partial 5-HT$_{1A}$ receptor agonist NAN 190 (0.03, 0.3 or 3.0 mg/kg, s.c., 15 min prior to testing) and activity was measured for 30 min [Experiment 1b].

Experiments 2a&b: Effect of systemic RU 24969 or CP 94253 administration on spontaneous and nicotine-induced locomotor activity

Groups of animals received randomized, counterbalanced series of four injections of either: vehicle + vehicle, vehicle + the mixed 5-HT receptor ligand RU 24969 (2.5 mg/kg, s.c., 10 mins prior), the 5-HT$_{1A/B}$ receptor antagonist pindolol (4.0 mg/kg, s.c., 30 min prior) + vehicle or pindolol + RU 24969 [Experiment 2a]; vehicle or the 5-HT$_{1B}$ receptor agonist CP 94253 (0.0625, 1.25 or 2.5 mg/kg, s.c., 15 min prior to testing) [Experiment 2b]. Locomotor activity was measured for 30 min and 60 min respectively.

Experiments 3a-c: Effect of systemic TFMPP administration on spontaneous and nicotine-induced locomotor activity

Groups of animals received a randomized, counterbalanced series of four injections of either: vehicle or the mixed 5-HT receptor agonist TFMPP (1.25, 2.5 or 5.0

mg/kg, s.c., 10 min prior) [Experiment 3a]; vehicle + vehicle, vehicle + TFMPP (3.0 mg/kg, s.c., 15 min prior), vehicle + the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg, s.c., 15 min prior) or TFMPP + WAY 100635 [Experiment 3b]; vehicle + vehicle, vehicle + TFMPP (3.0 mg/kg, s.c., 15 min prior), pindolol (4.0 mg/kg, s.c., 30 min prior) + vehicle or pindolol + TFMPP [Experiment 3c] and activity was measured for 30 min.

Experiments 4a-c: Effect of systemic 5-HT_{2C} receptor ligand administration on spontaneous and nicotine-induced locomotor activity

Groups of animals received a randomized, counterbalanced series of four injections of either: vehicle or the 5-HT_{2C} receptor agonist WAY 161503 (0.1, 0.3 and 1.0 mg/kg, s.c., 10 min prior) [Experiment 4a]; vehicle + vehicle, vehicle + WAY 161503 (1.0 mg/kg, sc, 10 min prior), the 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg, i.p., 30 min prior) + vehicle or SB 242084 + WAY 161503 [Experiment 4b]; vehicle + vehicle, vehicle + WAY 161503 (1.0 mg/kg, sc, 10 min prior), pindolol (4.0 mg/kg, sc, 15 min prior) + vehicle or WAY 161503 + pindolol [Experiment 4c] and activity was measured for 60 min.

Experiment 5a&b: Effects of intra-VTA CP 94253 or WAY 161503 on spontaneous and nicotine-induced hyperactivity

Groups of animals received a randomized, counterbalanced series of four injections of either: artificial cerebral spinal fluid (aCSF) or CP 94253 (0.625, 1.25 or 2.5

μg) [Experiment 5a]; aCSF or WAY 161503 (0.15, 0.5 or 1.5 μg) [Experiment 5b] and activity was measured for 60 min.

In both saline- and nicotine - treated animals, a positive control group was included to verify activity at the microinjection site. The animals received a systemic vehicle injection and either intra-VTA administration of aCSF, nicotine (12 μg) or the nAch receptor agonist cytisine (4 μg) and activity was measured for 60 min.

Statistical Analysis

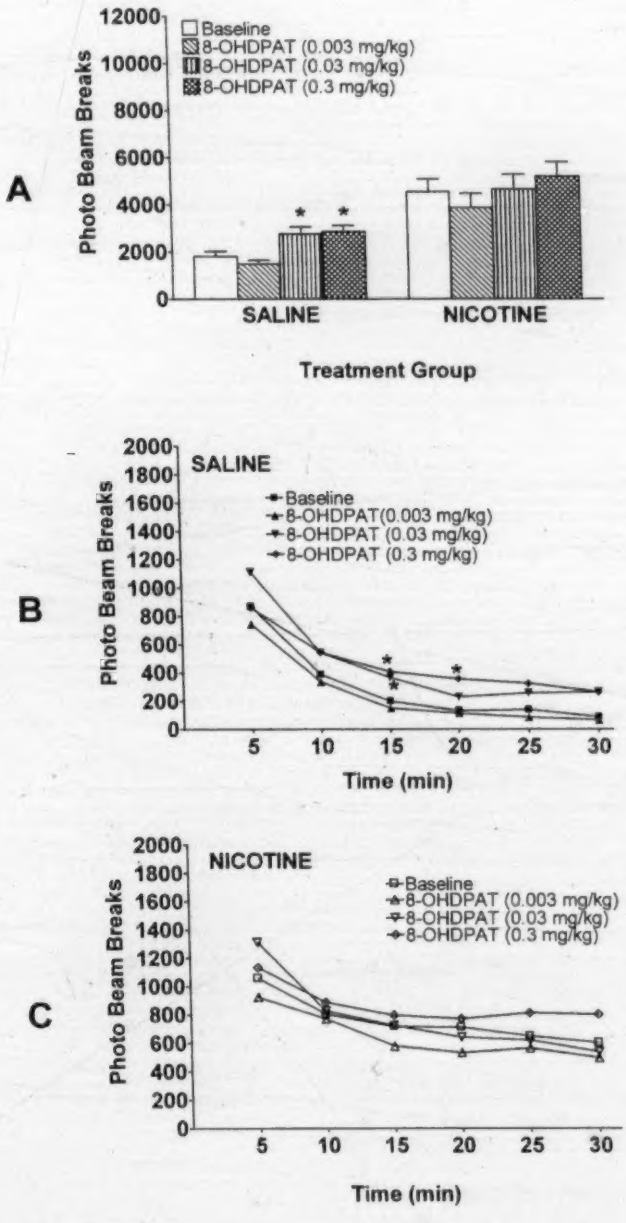
Experimental effects on locomotor activity were analyzed using a three-way (drug x time x group) or four-way (agonist x antagonist x time x group) repeated measures ANOVA. Drug treatment group was a between subjects factor. Where appropriate, analysis of time course data using a one-way repeated measures ANOVA across all drug groups at each 5 min interval was conducted. A significant F ratio ($P \leq 0.05$) on a 5 min interval was followed by comparison of each drug condition to vehicle treatment using Newman-Keul's post hoc tests ($\alpha=0.05$). To examine drug effects following intra-VTA microinjections of nicotine or cytisine, a paired samples t-test was performed between the nicotine or cytosine drug day and the day the animals received systemic administration of vehicle plus an intra-VTA injection of aCSF. Only results on horizontal locomotor activity counts are reported. All statistical analyses were completed using SPSS statistical software (SPSS 13.0, SPSS Inc., Chicago, U.S.A.).

Results

Experiment 1a&b: Effect of systemic 5-HT_{1A} receptor ligand administration on spontaneous and nicotine-induced locomotor activity

Experiment 1a: There was a significant effect of the 5-HT_{1A} receptor agonist 8-OHDPAT [F (2.666, 42.657) = 9.856, P>0.05], time [F (2.651, 42.422) = 140.984, P>0.05], group [F (1, 16) = 18.14, P>0.05], time x group interaction [F (2.651, 42.422) = 6.004, P>0.05] and 8-OHDPAT x time interaction [F (5.99, 89.577) = 3.393, P>0.05]. In animals pre-treatment with saline, 8-OHDPAT (0.03 and 0.3 mg/kg) was shown to increase total locomotor activity (Figure 5.1A). In addition, 8-OHDPAT (0.03 mg/kg) increased locomotor at 15 min, while 8-OHDPAT (0.3 mg/kg) increased locomotor activity at 15 and 20 min in saline-treated animals (Figure 5.1B). There was no significant effect of 8-OHDPAT in animals chronically treated with nicotine (P>0.05) (Figure 5.1C).

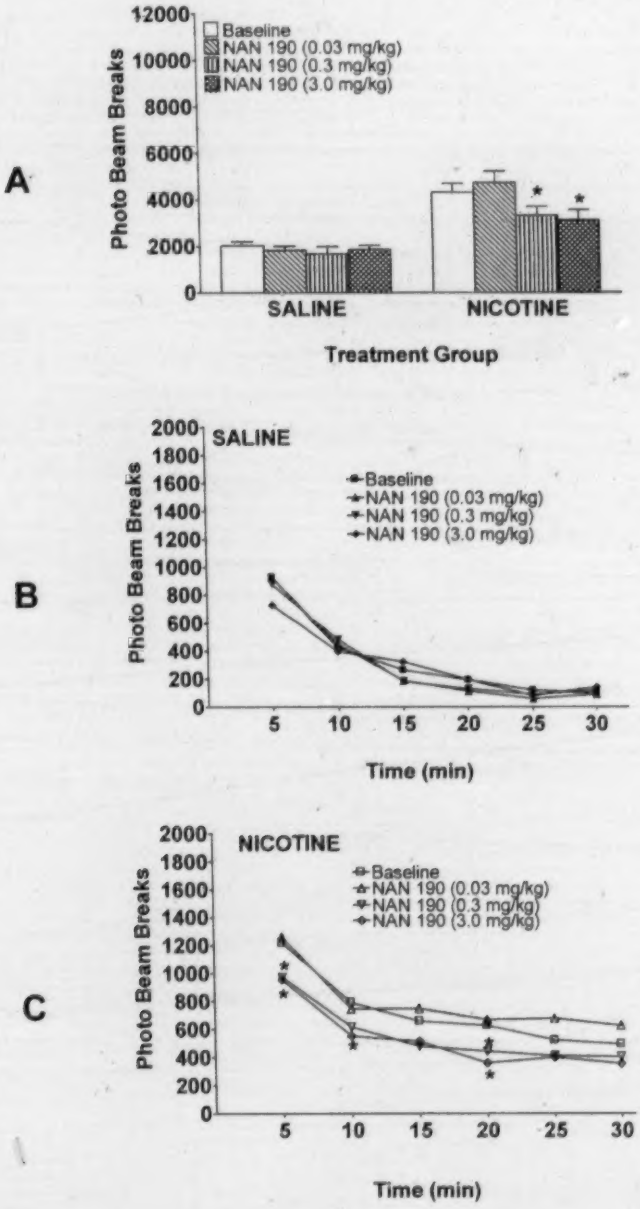
Figure 5.1. Effects of the 5-HT_{1A} agonist 8-OHDPAT (0.003 – 0.3 mg/kg) on total locomotor activity (A) and time course of changes in locomotor activity in saline-(B) and nicotine-(C) treated animals over 30 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 1b: There was a significant effect of the partial 5-HT_{1A} receptor agonist NAN 190 [$F(2.436, 38.976) = 9.297$, $P<0.05$], time [$F(3.30, 52.798) = 197.085$, $P<0.05$], group [$F(1, 16) = 22.676$, $P<0.05$], NAN 190 x group interaction [$F(2.436, 28.976) = 8.747$, $P<0.05$] and time x group interaction [$F(3.30, 52.798) = 3.387$, $P<0.05$].

In saline-treated animals NAN 190 had no effect on total locomotor activity; however, NAN 190 (0.3 and 3.0 mg/kg) decreased nicotine-induced hyperactivity (Figure 5.2A). NAN 190 did not affect spontaneous locomotor activity over the 30 min time period (Figure 5.2B). Local time course analysis revealed that NAN 190 (0.3 mg/kg) significantly reduced nicotine-induced hyperactivity at 5 and 20 min of testing. NAN 190 (3.0 mg/kg) decreased nicotine-induced hyperactivity for the first 10 min of testing and at 20 min (Figure 5.3C).

Figure 5.2. Effects of the partial 5-HT_{1A} receptor agonist NAN 190 (0.03 – 3.0 mg/kg) on total locomotor activity (A) and time course of changes in locomotor activity in saline- (B) and nicotine- (C) treated animals over 30 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.

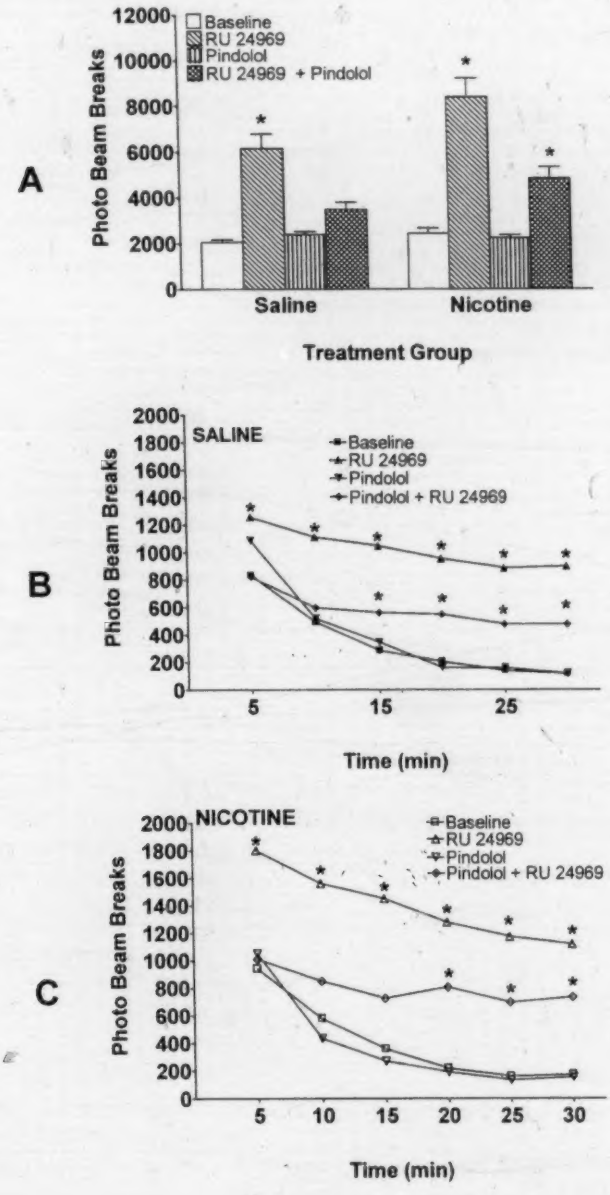


Experiments 2a&b: Effect of systemic RU 24969 or CP 94253 administration on spontaneous and nicotine-induced locomotor activity

Experiment 2a: There was a significant effect of RU 24969 [$F(1, 14) = 89.789$, $P<0.05$], pindolol [$F(1, 14) = 59.806$, $P<0.05$], time [$F(1.811, 25,355) = 108.903$, $P<0.05$], group [$F(1, 14) = 5.345$, $P<0.05$], RU 24969 x pindolol [$F(1, 14) = 53.874$, $P<0.05$] RU 24969 x group [$F(1, 14) = 5.408$, $P<0.05$], RU 24969 x time [$F(2.310, 32.34) = 23.211$, $P<0.05$], pindolol x time [$F(2.310, 39.736) = 3.477$, $P<0.05$], pindolol x time x group [$F(2.838, 39.736) = 3.297$, $P<0.05$] and RU 24969 x pindolol x time [$F(2.924, 40.934) = 9.533$, $P<0.05$].

RU 24969 was shown to increase total locomotor activity in saline- and nicotine-treated animals (Figure 5.3A). Local time course analysis in saline-treated animals revealed that RU 24969 increased locomotor activity for the total 30 min test period. Pretreatment with pindolol blocked the RU 24969-induced increase in locomotor activity in saline-treated animal for the first 10 min of testing (Figure 5.3B). In nicotine-treated animals, RU 24969 increased locomotor activity induced by nicotine. Pretreatment with pindolol blocked the enhancement of nicotine-induced hyperactivity by RU 24969 during the first 15 min of testing (Figure 5.3C). Administration of pindolol alone had no effect on local time course activity in either saline- or nicotine- treated animals.

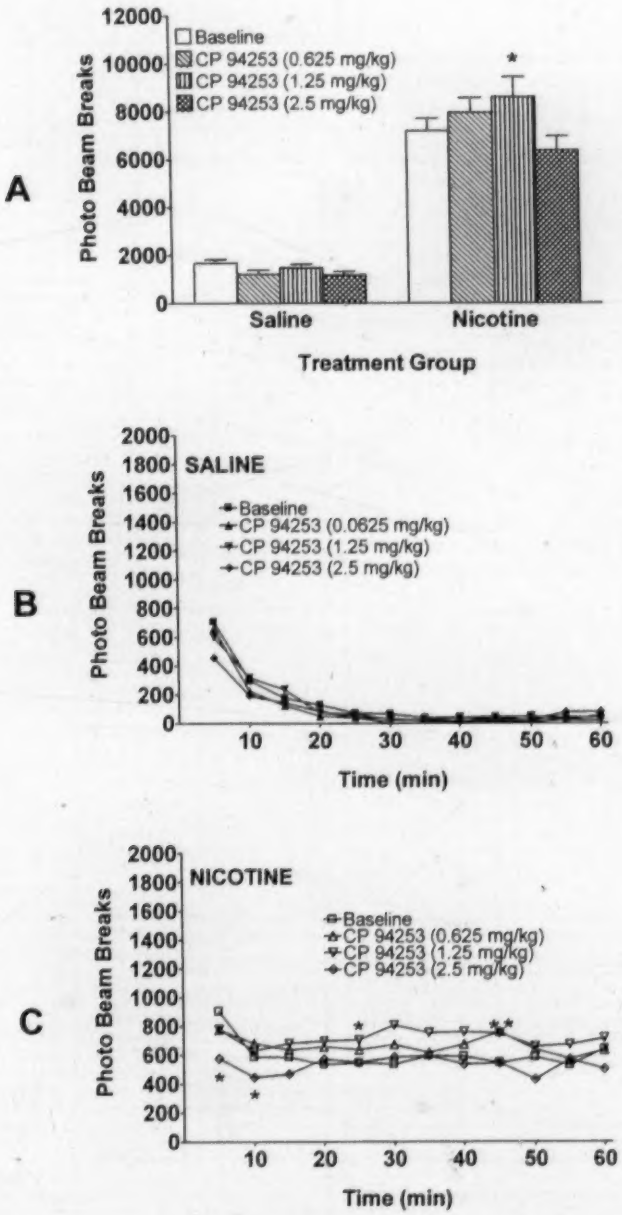
Figure 5.3. Effects of RU 24969 (2.5 mg/kg) and the 5-$HT_{1A/B}$ receptor antagonist pindolol (4.0 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 30 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 2b: There was a significant effect of the 5-HT_{1B} agonist CP 94253 [F (2.52, 35.286) = 8.692, P<0.05], time [F (4.191, 58.681) = 46.798, P<0.05] and group [F (1, 14) = 106.929, P<0.05]. There was also a significant CP 94253 x time interaction [F (2.346, 101.795) = 2.346, P<0.05], CP 94253 x group interaction [F (2.52, 35.286) = 6.864, P<0.05] and time x group interaction [F (4.191, 58.681) = 22.871, P<0.05].

CP 94253 (1.25 mg/kg) significantly increased nicotine-induced hyperactivity; however, stimulation of the 5-HT_{1B} receptor by CP 94253 did not significantly effect spontaneous locomotor activity (Figure 5.4A). During the first 10 min of testing, CP 94253 (2.5 mg/kg) decreased locomotor hyperactivity in nicotine-treated animals. At 25 and 45 min of testing, CP 94253 (1.25 mg/kg) increased nicotine induced hyperactivity; CP 94253 (0.0625 mg/kg) also increased nicotine-induced hyperactivity at 45 min (Figure 5.4C). CP 94253 had no effect on locomotor activity in saline-treated animals (Figure 5.4B).

Figure 5.4. Effects of the 5-HT_{1B} receptor agonist CP 94253 (0.0625 – 2.5 mg/kg) on total locomotor activity (A) and time course changes in locomotor activity in saline- (B) and nicotine-(C) treated animals over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.

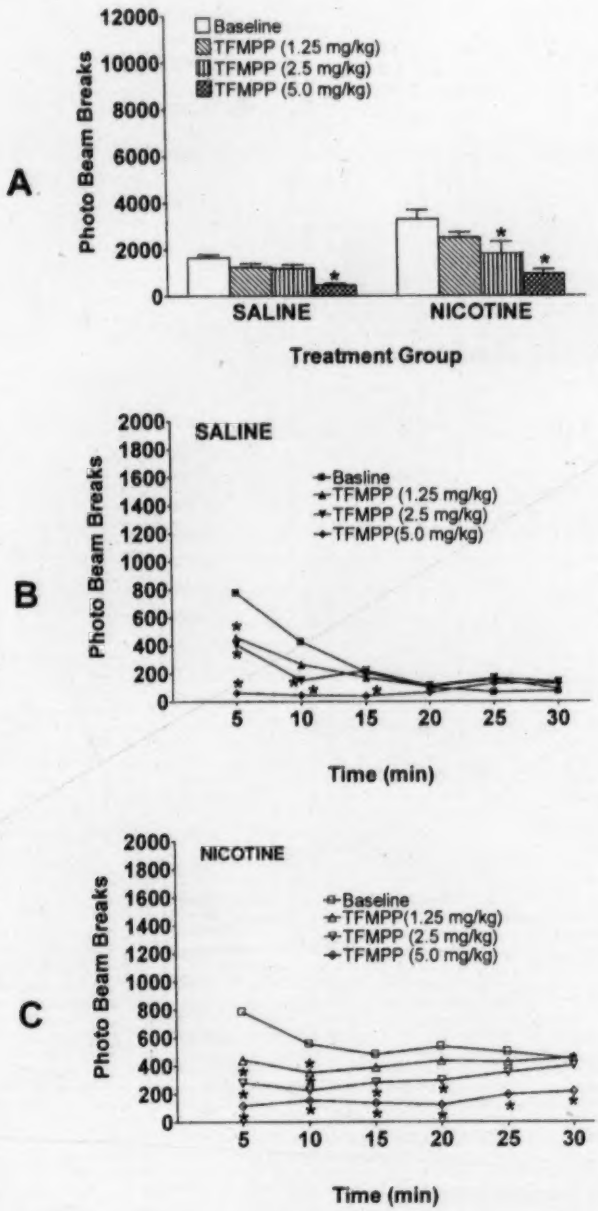


Experiments 3a-c: Effect of systemic TFMPP administration on spontaneous and nicotine-induced locomotor activity

Experiment 3a: There was a significant effect of TFMPP [$F(2.161, 34.570) = 26.223$, $P<0.05$], time [$F(2.348, 37.573) = 27.696$, $P<0.05$] and group [$F(1, 16) = 12.863$, $P<0.05$]. Significant interactions of TFMPP x group [$F(2.161, 34.57) = 3.583$, $P<0.05$], TFMPP x time [$F(26.099, 97.59) = 13.41$, $P<0.05$] time x group [$F(2.348, 37.573) = 17.107$, $P<0.05$] and TFMPP x time x group [$F(26.099, 97.59) = 2.542$, $P<0.05$] were also observed.

TFMPP (2.5 and 5.0 mg/kg; 5.0 mg/kg) significantly reduced nicotine-induced hyperactivity and spontaneous locomotor activity respectively (Figure 5.5A). Local time course analysis revealed that TFMPP (1.25 mg/kg) reduced locomotor activity during the first 5 min of testing; TFMPP (2.5 mg/kg and 5.0 mg/kg) reduced locomotor activity in saline-treated animals for the first 10 and 15 min of testing respectively (Figure 5.5B). TFMPP (1.25 mg/kg) reduced nicotine-induced hyperactivity for the first 10 min of testing; TFMPP (2.5 mg/kg) for the first 20 min of testing and TFMPP (5.0 mg/kg) for the entire 30 min testing period (Figure 5.5C).

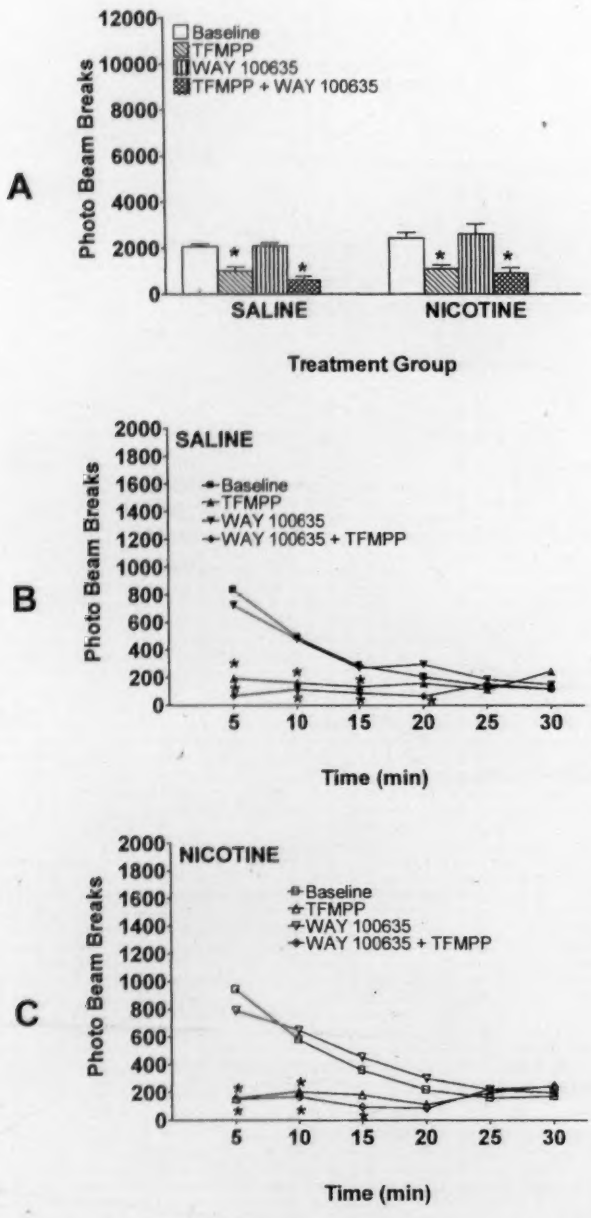
Figure 5.5. Effects of the mixed 5-HT receptor agonist TFMPP (1.25-5.0 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 30 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 3b: There was a significant effect of TFMPP [F (1, 14) = 140.842, P<0.05], time [F (3.572, 50.014) = 76.869, P<0.05], group [F (1, 14) = 1.807, P<0.05] and a significant TFMPP x time interaction [F (3.497, 48.963) = 56.953, P<0.05]. There was no significant effect of the 5-HT_{1A} receptor antagonist WAY 100635 or significant interaction of TFMPP x WAY 100635. TFMPP reduced locomotor activity in both saline- and nicotine- treated animals, this reduction in locomotor activity was not blocked by pretreatment with the 5-HT_{1A} receptor agonist WAY 100635 (Figure 5.6A).

Local time course analysis revealed that TFMPP and TFMPP + WAY 100635 significantly reduced locomotor activity in saline-treated animals for the first 20 and 15 min of testing respectively (Figure 5.6B). Pretreatment with TFMPP or TFMPP + WAY 100635 significantly reduced nicotine-induced hyperactivity over the first 15 min of testing (Figure 5.6C).

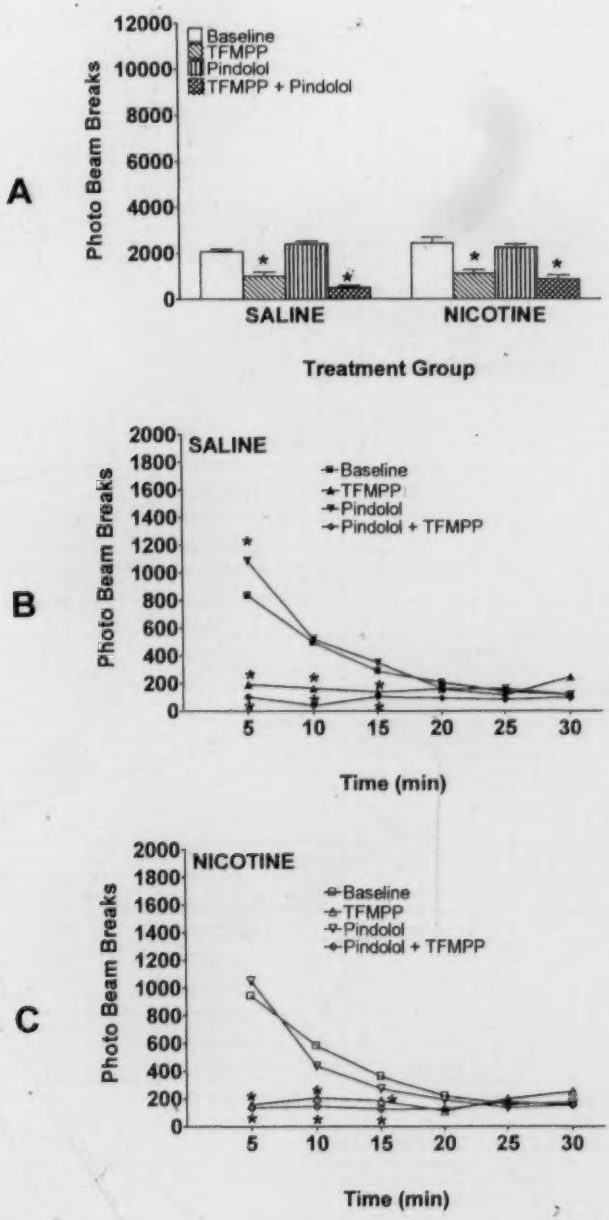
Figure 5.6. Effects of TFMPP (3.0 mg/kg) and the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 30 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 3c: TFMPP decreased spontaneous and nicotine-induced locomotor activity [$F(1, 14) = 357.653$, $P<0.05$]. There was also a significant effect of time [$F(2.754, 38.559) = 89.806$, $P<0.05$] and group [$F(1, 14) = 0.999$, $P<0.05$]. Significant interactions of TFMPP x time [$F(2.492, 34.89) = 117.125$, $P<0.05$], pindolol x time [$F(3.238, 45.326) = 4.483$, $P<0.05$], TFMPP x pindolol x time [$F(3.588, 50.266) = 3.298$, $P< 0.05$] were also observed (Figure 5.7A).

TFMPP or TFMPP + pindolol significantly reduced locomotor activity during the first 15 min of testing in saline-treated animals. In addition, pindolol increased spontaneous locomotor activity during the first 5 min of testing (Figure 5.7B). In animals treated with nicotine, TFMPP or TFMPP + pindolol reduced nicotine-induced hyperactivity during the first 10 min of testing (Figure 5.7C).

Figure 5.7. Effects of TFMPP (3.0 mg/kg) and the 5-$HT_{1A/B}$ receptor antagonist pindolol (4.0 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 30 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.

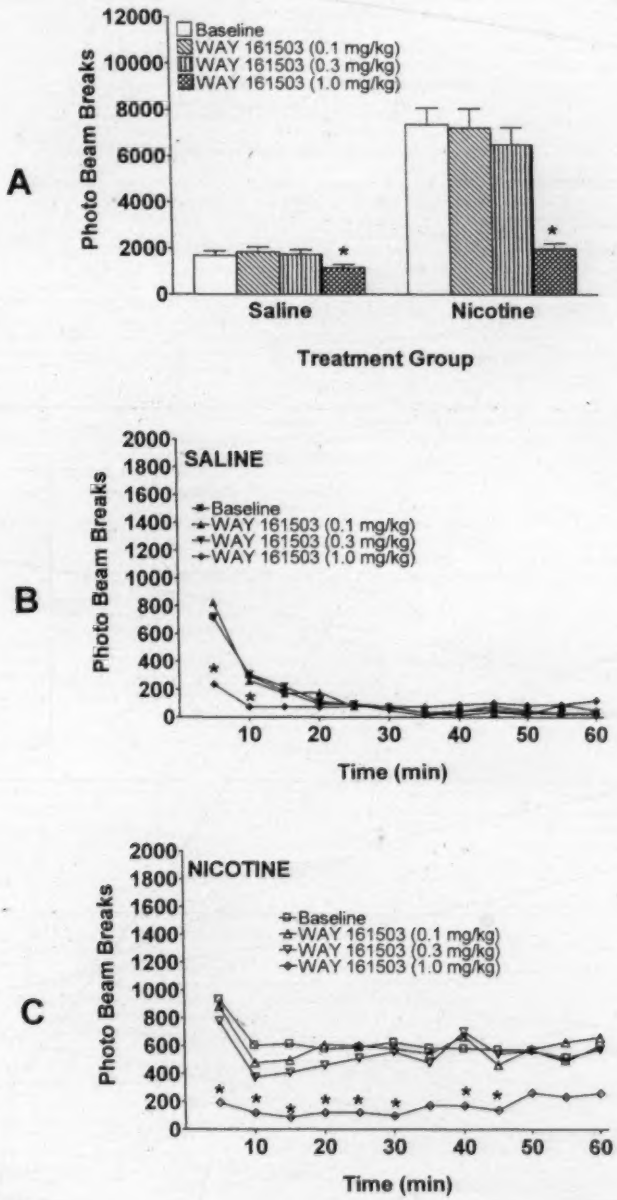


Experiments 4a-c: Effect of systemic 5-HT_{2C} receptor ligand administration on spontaneous and nicotine-induced locomotor activity

Experiment 4a: WAY 161503 (1.0 mg/kg) [$F(1.525, 21.352) = 39.73$, $P < 0.05$] significantly reduced spontaneous locomotor activity as well as nicotine-induced hyperactivity (Figure 5.8A). There was also a significant effect of time [$F(5.506, 77.083) = 50.008$, $P < 0.05$] and group [$F(1, 14) = 50.585$, $P < 0.05$]. Significant interactions of WAY 161503 x group [$F(1.525, 21.352) = 25.227$, $P < 0.05$], time x group [$F(5.506, 77.083) = 15.568$, $P < 0.05$] and WAY 161503 x time [$F(5.541, 77.578) = 4.974$, $P < 0.05$] were also observed. Local time course analysis revealed that WAY 161503 (1.0 mg/kg) reduced locomotor activity in saline-treated animals during the first 10 min of testing (Figure 5.8B). WAY 161503 (1.0 mg/kg) significantly reduced nicotine-induced hyperactivity during the first 30 min of testing and at 40 and 45 min (Figure 5.8C).

Figure 5.8. Effects of the 5-HT_{2C} receptor agonist WAY 161503 (0.01 – 1.0 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 60 min. Data shown are means ± S.E.M.

* Significant $P<0.05$, relative to control.

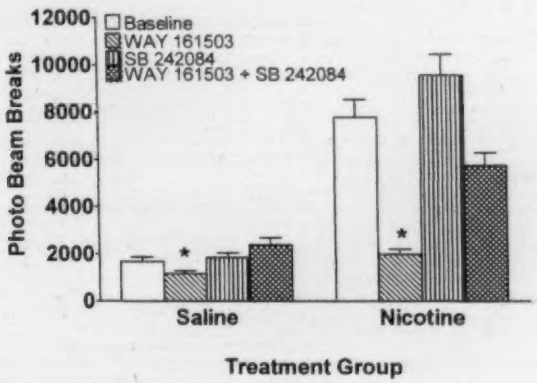


Experiment 4b: WAY 161503 significantly reduced total locomotor activity in animals treated with saline or nicotine [$F(1, 14) = 39.925$, $P < 0.05$]. There was also a significant effect of SB 242084 [$F(1, 14) = 93.308$, $P<0.05$], time [$F(4.048, 56.679) = 35.431$, $P<0.05$] and group [$F(1,14) = 80.981$, $P<0.05$]. Significant interactions of WAY 161503 x SB 242084 [$F(1, 14) = 19.125$, $P<0.05$], WAY 161503 x group [$F(1,14) = 36.23$, $P<0.05$], SB 242084 x time [$F(1, 14) = 33.306$, $P<0.05$], time x group [$F(4.048, 56.679) = 7.168$, $P<0.05$], WAY 161503 x time [$F(4.768, 66.751) = 8.511$, $P<0.05$], SB 242084 x time [$F(5.555, 77.769) = 2.164$, $P<0.05$] and WAY 161503 x SB 242084 x time [$F(4.449, 62.283) = 2.566$, $P<0.05$] were also observed (Figure 5.9A).

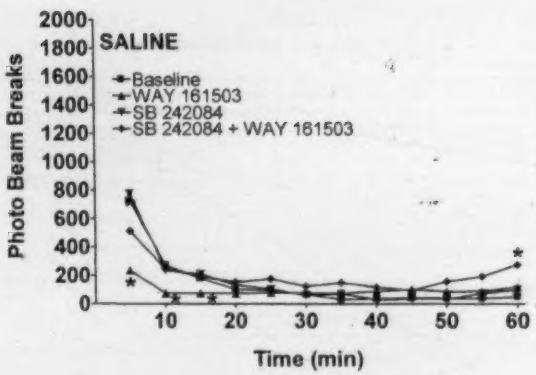
Local time course analysis revealed that WAY 161503 reduced locomotor activity during the first 15 min of testing. SB 242084 blocked the reduction in locomotor activity induced by WAY 161503 with the exception of the first 5 min of testing; SB 242084 + WAY 161503 was significantly different than control treatment at the 60 min time point. SB 242084 did not significantly affect locomotor activity in saline-treated animals (Figure 4B). Nicotine-induced hyperactivity was reduced by WAY 161503 for the first 35 min of testing as well as during the 45 and 55 min test periods. SB 242084 blocked the reduction in nicotine- induced hyperactivity by WAY 161503 with the exception of the 15, 20 and 30 min test points. Administration of SB 242084 did not significantly affect nicotine-induced hyperactivity (Figure 5.9C).

Figure 5.9. Effects of WAY 161503 (1.0 mg/kg) and the 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.

A



B



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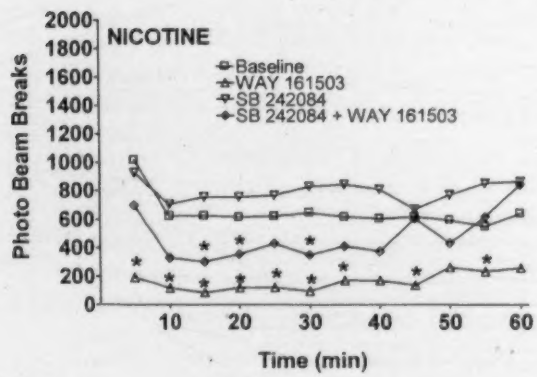
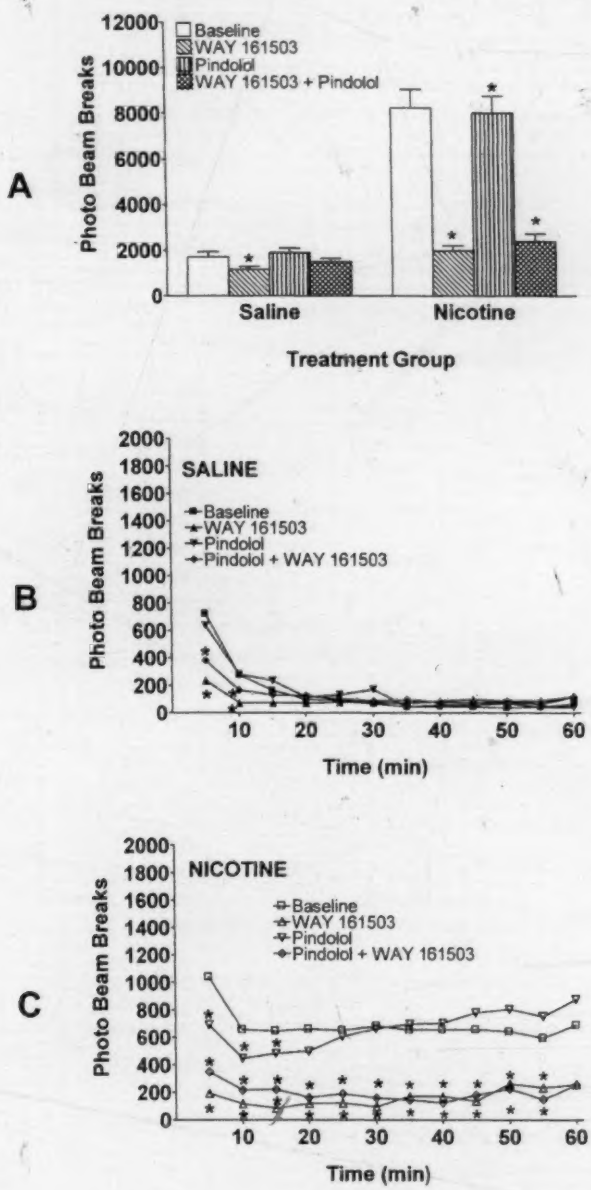


Experiment 4c: WAY 161503 [F (1, 14) = 75.593, P<0.05] significantly reduced total locomotor activity in animals treated with either saline or nicotine. A significant effect of time [F (4.895, 68.528) = 43.332, P<0.05], group [F (1, 14) = 62.505, P<0.05] as well as significant WAY 161503 x group [F (1, 14) = 55.243, P<0.05], time x group [F (4.895, 68.528) = 18.727, P<0.05], WAY 161503 x time [F (3.898, 54.577) = 8.265, P<0.05], WAY 161503 x time x group [F (3.898, 54.577) = 7.271, P<0.05], pindolol x time x group [F (5.012, 70.17) = 2.954, P<0.05], WAY 161503 x pindolol x time [F (5.134, 71.883) = 10.087, P<0.05] and WAY 161503 x pindolol x time x group [F (5.134, 71.883) = 4.262, P<0.05] interactions were also observed. Following Newman-Keul's post hoc test it was revealed that pindolol + WAY 161503 was significantly different than control treatment in saline-treated animals. In nicotine-treated animals, WAY 161503 or pindolol + WAY 161503 decreased total locomotor activity compared to control (Figure 5.10A).

WAY 161503 or pindolol + WAY 161503 significantly reduced spontaneous locomotor activity for the first 10 min of testing (Figure 5.10B). WAY 161503 or pindolol + WAY 161503 significantly reduced nicotine-induced hyperactivity for the first 55 min of testing; pindolol was also found to be significantly lower than baseline for the first 15 min of testing (Figure 5.10C).

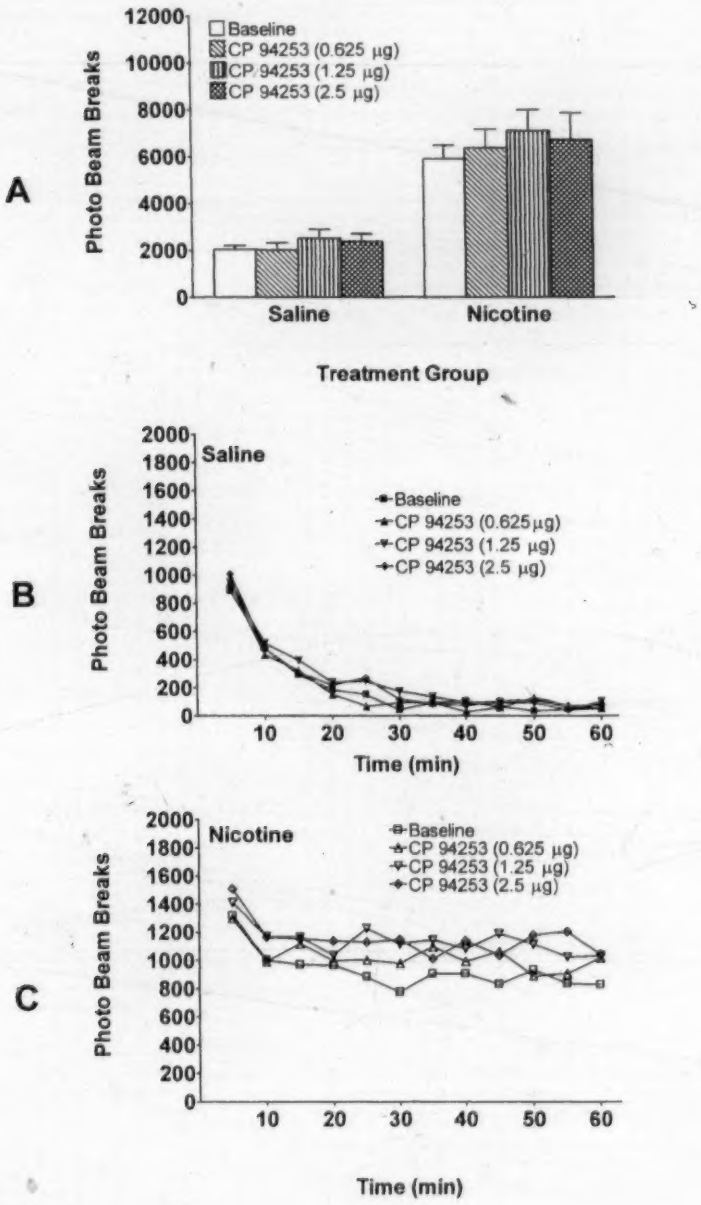
Figure 5.10. Effects of WAY 161503 (1.0 mg/kg) and the 5-$HT_{1A/B}$ receptor antagonist pindolol (4.0 mg/kg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 5a&b: Effects of intra-VTA CP 94253 or WAY 161503 on spontaneous and nicotine-induced hyperactivity

Experiment 5a: 3-way repeated measures ANOVA revealed a significant effect of CP 94253 [$F(2.929, 49.788) = 4.222$, $P<0.05$], time [$F(2.636, 44.805) = 43.568$, $P<0.05$], group [$F(1, 17) = 69.507$, $P<0.05$], and a significant time x group interaction [$F(2.636, 44.805) = 10.642$, $P<0.05$]. Post-hoc analysis found that microinjection of CP 94253 (0-2.5 μg) into the VTA did not alter total locomotor activity in either saline- or nicotine-treated animals (Figure 5.11A-C).

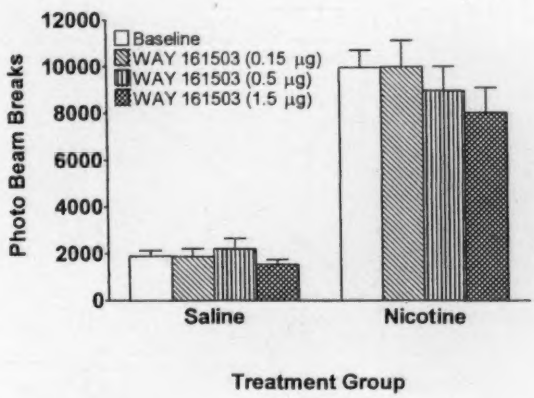
Figure 5.11. Lack of effect of intra-VTA administration of the 5-HT_{1B} receptor agonist CP 94253 (0.625-2.5 μg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.



Experiment 5b: Intra-VTA microinjection of WAY 161503 (0-1.5 μg) did not effect locomotor activity in either saline- or nicotine- treated animals. There was however a significant effect of time [$F(4.302, 60.224) = 34.693$, $P<0.05$], group [$F(1, 14) = 55.881$, $P<0.05$] and time x group [$F(4.302, 60.224) = 7.13$, $P<0.05$] interaction (Figure 5.12A-C).

Figure 5.12. Lack of effect following intra-VTA administration of the 5-HT_{2C} receptor agonist WAY 161503 (0.15 – 1.5 µg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals over 60 min. Data shown are means ± S.E.M.

A



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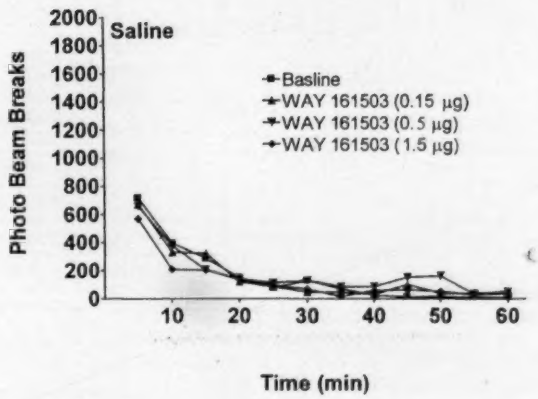

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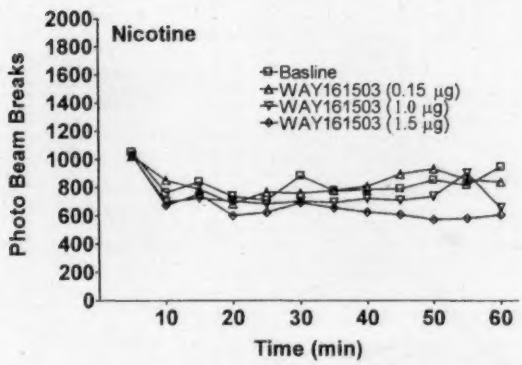


Intra-VTA nicotine injections did not alter locomotor activity in saline- or nicotine-treated animals, however intra-VTA cytisine injections caused an increase in locomotor activity in saline- treated [$t(4) = 3.192$, $P < 0.05$] but not nicotine-treated animals (Figure 5.13A). Local time course analysis revealed that intra-VTA nicotine administration decreased spontaneous locomotor activity for the first five minutes of testing; whereas intra-VTA infusion of cytisine increased spontaneous locomotor activity during the first 5 min of testing and 15-35 min test period (Figure 5.13B). Intra-VTA administration of nicotine increased locomotor activity compared to intra-VTA aCSF administration in nicotine-treated animals at the 55 min time point. Intra-VTA administration of cytisine did not affect locomotor activity in nicotine-treated animals (Figure 5.13C).

Figure 5.13. Effects of intra-VTA microinjection of nicotine (12 μg) or cytisine (4 μg) on total locomotor activity (A) and time course changes on locomotor activity in saline- (B) and nicotine- (C) treated animals recorded over 60 min. Data shown are means ± S.E.M. * Significant $P<0.05$, relative to control.

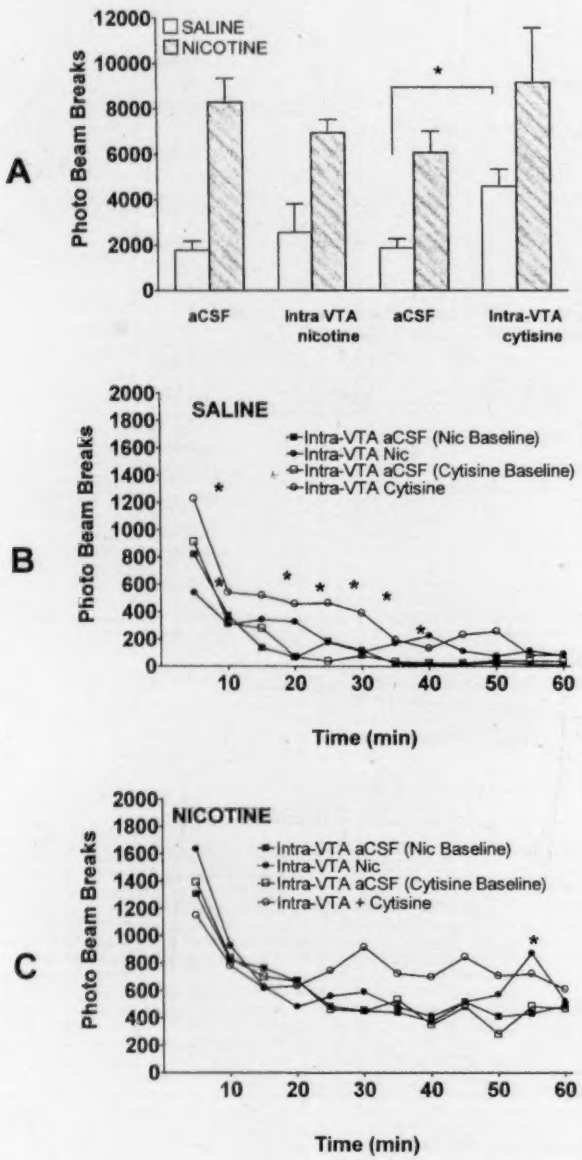
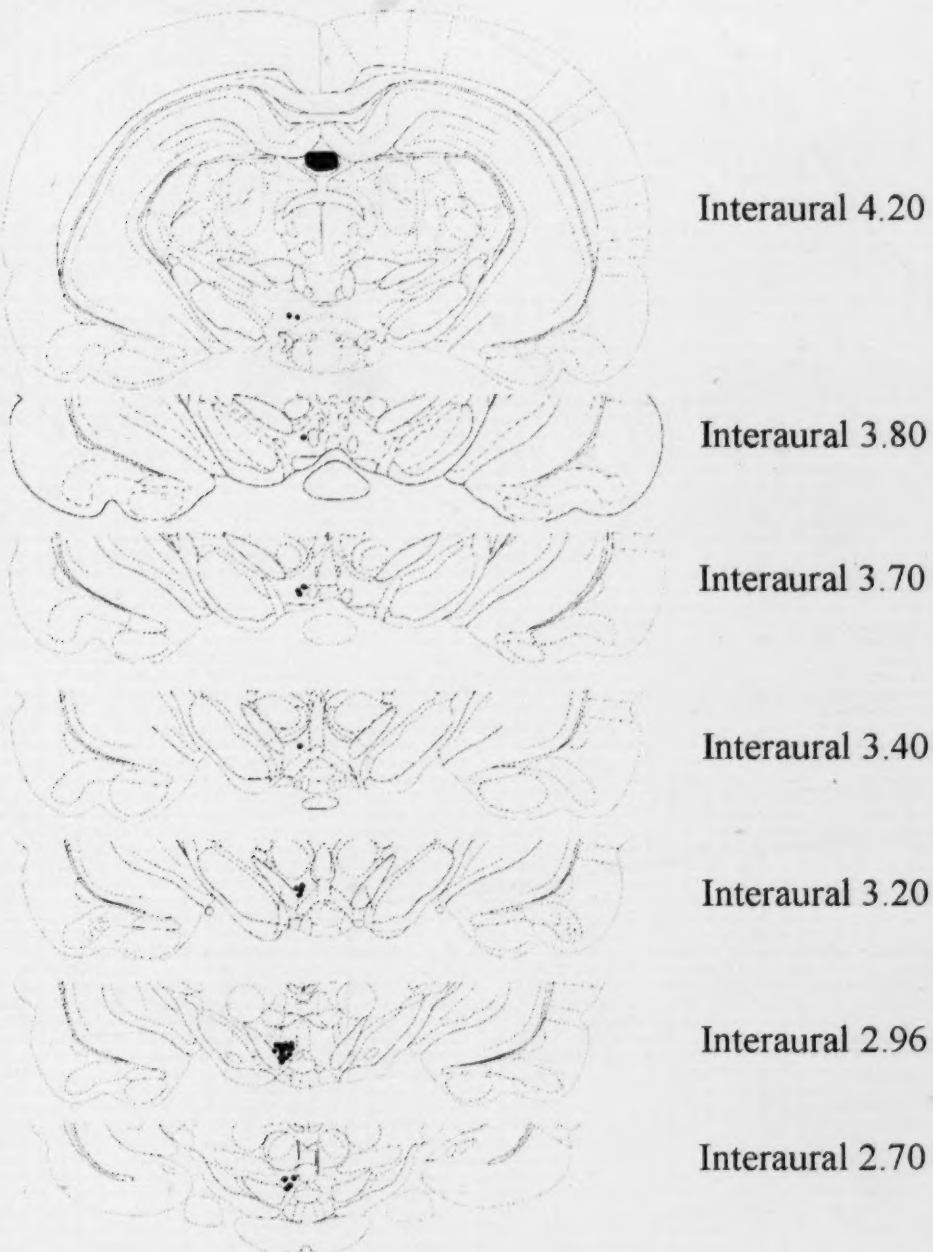


Figure 5.14. Illustration of histological verification of cannula sites in the VTA. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).



Note: Experiments with WAY 161503 and CP 94253 were conducted by T Mosher; all other experiments were conducted by S Waddock and have been reported in thesis form Waddock (1997). In Sarah Waddock's (1997) thesis, the data was reported as a percentage of control. The representation of the data were presented in my thesis consists of averages of raw data and in comparison to my experiments, the effect of nicotine on locomotor activity appears to be small. Nonetheless, the statistics on the data presented by Sarah Waddock (1997) (percent of control) and my presentation of raw data both correlate to a significant effect of nicotine by ANOVA and significant drug x nicotine interactions. In addition, the Waddock data were recorded over a 30 min period whereas the data obtained from my experiments were recorded over 60 min. The majority of the vehicle activity took place in the first 30 min, thus, the appearance of the nicotine effect during that time period appears to be reduced.

Discussion

The purpose of the present study was to examine the effect of agonists which act at the 5-$HT_{1A/B/2C}$ receptors on spontaneous locomotor activity and nicotine-induced hyperactivity. These receptor subtypes were chosen as they are known to alter mesocorticolimbic DA function; an effect which mediates nicotine-induced hyperactivity (Benwell and Balfour, 1992; Benwell et al., 1995; Clarke et al., 1988; Imperato et al., 1986; Nisell et al., 1994a). For example, systemic administration of 5-HT_{1A} receptor agonists have been reported to increase DA concentrations in the medial PFC (Arborelius et al., 1993b; Diaz-Mataix et al., 2005; Nakayama et al., 2004; Tanda et al., 1994) and VTA (Diaz-Mataix et al., 2005); inconsistent results have been reported on NAS DA

concentrations (Arborelius et al., 1993b; Boulenguez et al., 1996; Jiang et al., 1990; Tanda et al., 1994). In contrast, intra-DRN administration of the 5-HT_{1A} receptor agonist 8-OHDPAT (Yoshimoto and McBride, 1992) but not intra-VTA administration (Guan and McBride, 1989) may decrease extracellular DA levels in the NAS. The firing rate and burst activity of VTA DA neurons is also increased following systemic or intra-VTA administration of 5-HT_{1A} receptor agonists (Arborelius et al., 1993a; Arborelius et al., 1993b; Diaz-Mataix et al., 2005; Lejeune and Millan, 1998; Millan et al., 1997). Intra-medial PFC administration of the 5-HT_{1A} receptor agonist BAY x 3702 may increase extracellular DA concentrations at low doses, and reduced it at higher doses in this area (Diaz-Mataix et al., 2005). Blockade of the 5-HT_{1A} receptor by WAY 100635 had no effect on DA concentrations in the medial PFC (Millan et al., 1997; Nakayama et al., 2004), NAS (Yan and Yan, 2001) or VTA DA neuronal firing (Diaz-Mataix et al., 2005; Lejeune and Millan, 1998).

Within this study, activation of the 5-HT_{1A} receptor by systemic administration of the 5-HT_{1A} receptor agonist 8-OHDPAT increased locomotor activity in animals treated with saline but not nicotine. These results are consistent with those of Olausson (2001) who reported that co-administration of 8-OHDPAT increased locomotor activity following acute but not chronic nicotine treatment. One explanation for this may be due to a decrease in 5-HT release following 5-HT_{1A} autoreceptor stimulation which may disinhibit DA release. This may occur via a 5-HT_{1A} receptor mediated feedback inhibition in the DRN or MRN. Although an increase in locomotor activity was expected following administration of 8-OHDPAT in nicotine-treated animals, this effect was not observed. This may not be to a ceiling effect on locomotor activity in these animals as

administration of either CP 94253 or RU 24969 was demonstrated to increase nicotine-induced hyperactivity. In contrast to 8-OHDPAT, the partial 5-HT_{1A} receptor agonist NAN 190 [high affinity for the 5-HT_{1A} receptor; may also have an affinity for the 5-HT_{2A} and α_1 adrenergic receptors] (Glennon et al., 1988a; Paluchowska et al., 1999) did not affect spontaneous locomotor activity, but attenuated nicotine-induced hyperactivity. As NAN 190 is also known to act as an antagonist at post-synaptic 5-HT_{1A} receptors (Hjorth and Sharp, 1990; Przegalinski et al., 1990), this may account for the differential functional results of NAN 190 and of 8-OHDPAT in the present study. This result is consistent with the observation of 8-OHDPAT and NAN 190 having differential effects in other behavioural tests. In drug-discrimination learning experiments, where animals are trained to discriminate between 8-OHDPAT and saline, NAN 190 blocked the 8-OHDPAT cue (Barrett and Gleeson, 1992; Filip and Przegalinski, 1996; Glennon et al., 1988b; Kleven and Koek, 1998; Schreiber and de Vry, 1993; Schreiber et al., 1995). 8-OHDPAT generalizes to the discriminative stimulus cue of the mixed 5-HT receptor agonist indorenate, however, NAN 190 reduced the discriminative index of indorenate by 50 % (Sanchez and Velazquez-Martinez, 2001). In addition, NAN 190 blocked the decrease in locomotor activity induced by intra-NAS administration of 8-OHDPAT (Plaznik et al., 1994). These studies support a partial antagonist action of NAN 190 at the 5-HT_{1A} receptor. By contrast, the selective 5-HT_{1A} receptor antagonist WAY 100635 did not alter nicotine-induced hyperactivity or spontaneous locomotor activity in the present study. In addition to action at the 5-HT_{1A} receptor, NAN 190 also acts as an antagonist at the adrenergic α_1 receptor. Blockade of adrenergic α_1 receptors by prazosin may not effect locomotor activity (Wellman et al., 2002), however prazosin attenuated

the locomotor increasing effects induced by nicotine (Suemaru et al., 1994), cocaine (Wellman et al., 2002), morphine (Drouin et al., 2001) and AMPH (Blanc et al., 1994; Dickinson et al., 1988). Thus, the effects of NAN 190 on nicotine-induced hyperactivity may be due to blockade of adrenergic α_1 receptor or to actions on multiple receptor subtypes.

Blockade of the 5-HT_{1A} receptor by WAY 100635 did not alter locomotor activity in the present study. This is not supportive of a tonic 5-HT_{1A} autoreceptor feedback as a factor in the regulation of DA release. 5-HT_{1A} receptor-mediated changes in locomotor activity observed following administration of 5-HT_{1A} receptor agonists may be due to differential sites of action (i.e. pre- vs. post-synaptic). If the effects on locomotor activity following administration of 5-HT_{1A} receptor ligands are mediated at presynaptic 5-HT_{1A} receptor binding sites, then blocking these receptors with a silent antagonist such as WAY 100635 may prevent 5-HT inhibitory feedback; an effect that may decrease DA activity via an increase in 5-HT inhibition. In contrast, if the 5-HT_{1A} receptor-mediated effects on locomotor activity are mediated via action at post-synaptic 5-HT_{1A} receptors, blockade of these receptors by administration of WAY 100635 may not alter locomotor activity as there may be no effect on 5-HT release (Waddock, 1997).

Similar to stimulation of 5-HT_{1A} receptors, activation of 5-HT_{1B} receptors increase mesocorticolimbic extracellular DA levels. Systemic or intra-ventral subicular area administration of the mixed 5-HT receptor agonist (5-$HT_{1A/B/2C}$) RU 24969 may increase DA levels with the NAS shell (Boulenguez et al., 1996). RU 24969 was also found to increase the concentration of DA in the striata when injected into this area (Benloucif and Galloway, 1991). In addition, intra-VTA administration of selective 5-

HT_{1B} receptor agonists increase extracellular DA concentrations in both the VTA and the ipsilateral NAS (Yan and Yan, 2001; Yan et al., 2004); however, conflicting results have been reported (Yan et al., 2005). Administration of selective 5-HT_{1B} receptor agonists were also reported to increase extracellular DA levels in the striatum, PFC and NAS when injected into these regions (Galloway et al., 1993; Iyer and Bradberry, 1996; Yan and Yan, 2001). In general, stimulation of the 5-HT_{1B} receptor may enhance mesocorticolimbic DA levels.

In the present study, systemic but not intra-VTA administration of the 5-HT_{1B} receptor agonist CP 94253 (1.25 mg/kg) increased nicotine-induced hyperactivity; no effect on spontaneous locomotor activity was observed. The results of the current study are in agreement with previous studies which reported that administration of CP 94253 may not effect locomotor activity except at a very high does (32.0 mg/kg) (Koe et al., 1992; Lin and Parsons, 2002). Previously, CP 94253 was found to increase both AMPH- and cocaine- induced hyperactivity (Przegalinski et al., 2001a; Przegalinski et al., 2001b). These results indicate that systemic administration of 5-HT_{1B} receptor agonists may increase the locomotor effects of psychostimulants. Within the mesocorticolimbic system, intra-PFC administration of CP 94253 increased DA levels in this area (Iyer and Bradberry, 1996); intra-VTA administration of this compound may not alter DA concentrations in the NAS alone, but did enhance the increase in NAS DA release induced by systemic administration of ethanol (Yan et al., 2005). It was hypothesized that intra-VTA administration may increase nicotine-induced hyperactivity. Administration of CP 94253 into the VTA did not affect nicotine-induced hyperactivity; indicating that stimulation of 5-HT_{1B} receptors in the VTA may not be responsible for the

increase in nicotine-induced locomotor activity observed following systemic administration of this compound. Although local infusion of CP 94253 into the VTA did not alter locomotor activity, the injection site was active as demonstrated by an increase in spontaneous locomotor activity by intra-VTA administration of the nAch receptor agonist cytisine; this result is consistent with previous reports (Museo and Wise, 1990, 1994, 1995; Reavill and Stolerman, 1990).

The mixed 5-HT receptor agonist RU 24969 induced an increase in locomotor activity in both saline- and nicotine- treated animals; this effect was attenuated by pretreatment with the 5-$HT_{1A/B}$ receptor antagonist pindolol. These results lend support to the hypothesis of 5-HT inhibition of DA activity and indicate that it may be regulated in part by 5-HT_{1B} autoreceptor feedback (Waddock, 1997). Previously, the 5-HT_{1B} receptor has been implicated in behaviours induced by RU 24969. For example, RU 24969 was able to extinguish cocaine seeking behaviour and decrease ICSS reward thresholds, effects which were reversed by the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (Acosta et al., 2005; Harrison et al., 1999). Intra-NAS infusion of RU 24969 reduced responding for AMPH self-administration an effect which was similar to that of intra-NAS administration of the 5-HT_{1B} receptor agonist CP 93129 (Fletcher et al., 2002). RU 24969-induced locomotor hyperactivity has been attributed to activation of both the 5-HT_{1A} and the 5-HT_{1B} receptor subtypes (Martinez-Price and Geyer, 2002; O'Neill and Parameswaran, 1997). However, conflicting results have been reported as RU 24969-induced hyperactivity was blocked by WAY 100635 but not GR 127935 (Kalkman, 1995). Thus, the effect RU 24969 on locomotor activity observed in the present experiment may be due to stimulation of both the 5-HT_{1A} and 5-HT_{1B} receptors as

stimulation of RU 24969 does not have the same pattern of activity as stimulation of 5-HT_{1A} or 5-HT_{1B} receptors alone as demonstrated by experiments with 8-OHDPAT and CP 94253 respectively.

In contrast to activation of 5-HT autoreceptors, stimulation of the post-synaptic 5-HT_{2C} receptors negatively modulate the activity of mesocorticolimbic DA neurons. Stimulation of 5-HT_{2C} receptors by systemic administration of the mixed 5-HT receptor (with action at the 5-HT_{2C} receptor) agonists Ro 60-0175, MK 212 and mCPP (but not the 5-$HT_{2A/C}$ receptor agonist DOI) decreased DA release in the NAS, frontal cortex and reduced the firing rate of VTA DA neurons (Di Giovanni et al., 2000; Di Matteo et al., 1999, 2000; Gobert et al., 2000; Millan et al., 1998). In addition, nicotine-induced increases in NAS DA release and VTA DA neuron firing was reduced by administration of Ro 60-0175 (Di Matteo et al., 2004; Pierucci et al., 2004). Intra-VTA administration of the mixed 5-HT receptor agonist (5-$HT_{1A/B/2C}$) TFMPP did not affect extracellular DA levels in the ipsilateral NAS (Guan and McBride, 1989), although intra-NAS infusion of the 5-$HT_{2A/C}$ receptor agonist DOI decreased extracellular DA concentrations in this area (Yan, 2000). In addition, the mixed 5-HT receptor agonists TFMPP and mCPP increased extracellular DA concentrations in the striatum; however this may be due to their effects on other receptors such as the 5-HT_{1B} receptor (Benloucif and Galloway, 1991). In contrast to 5-$HT_{1A/B}$ receptor stimulation, activation of the 5-HT_{2C} receptor decreases mesocorticolimbic DA activity.

The mixed 5-HT receptor ligand TFMPP may alter locomotor activity via stimulation of the 5-HT_{2C} receptor (Lucki et al., 1989; Mosher et al., 2005). In the current study, pretreatment with either TFMPP or the selective 5-HT_{2C} receptor agonist

WAY 161503 significantly reduced spontaneous locomotor activity and nicotine-induced hyperactivity. The reduction in locomotor activity following TFMPP administration was unaffected by pretreatment with the 5-$HT_{1A/B}$ receptor antagonist pindolol, indicating that this effect may be mediated by an action at 5-HT_{2C} receptors. The effects of TFMPP and of the selective 5-HT_{2C} receptor agonist WAY 161503 on locomotor activity were similar; WAY 161503 reduced locomotor activity in both saline- and nicotine- treated animals. The effect of WAY 161503 was not blocked by pretreatment with pindolol, but was decreased by the selective 5-HT_{2C} receptor antagonist SB 242084. These results are consistent with previous reports of the 5-$HT_{2A/C}$ receptor agonist DOI reducing the locomotor stimulant effects of nicotine following chronic nicotine treatment (Olausson et al., 2001) as well as reversing the decrease in locomotor activity observed following acute nicotine administration (Batman et al., 2005). These results are also consistent with Grottick et al. (2001) reporting that the 5-HT_2 receptor agonist Ro 60-0175 decreased both nicotine-induced hyperactivity and nicotine self-administration. As nicotine-induced hyperactivity is known to involve increases in DA transmission within with mesocorticolimbic system (Benwell and Balfour, 1992; Benwell et al., 1995; Clarke et al., 1988; Damsma et al., 1989; Imperato et al., 1986; Rahman et al., 2003), and 5-HT_{2C} receptor activation decreases dopamine activity in this system, it is possible that the decrease in locomotor activity observed may be due a reduction in mesocorticolimbic DA release following 5-HT_{2C} receptor stimulation.

Previous reports have demonstrated that activation of 5-HT_{2C} receptors within the mesocorticolimbic system decreased both extracellular DA concentrations and DA neuronal activity most likely via activation of 5-HT_{2C} receptors located on GABA

containing neurons. (Di Giovanni et al., 2000; Di Giovanni et al., 2001; Di Matteo et al., 2000; Di Matteo et al., 2001; Di Matteo et al., 2002; Di Matteo et al., 2004; Millan et al., 1998; Pozzi et al., 2002). Thus, infusion of WAY 161503 into this system may decrease nicotine-induced hyperactivity via a reduction in DA release. This hypothesis was examined by microinjection of WAY 161503 into the VTA, but this was found to have no effect on locomotor activity in either saline- or nicotine-treated animals. This result may indicate that activation of 5-HT_{2C} receptors within the VTA does not affect nicotine-induced behaviours. To the author's knowledge, this is the first report of intra-VTA administration of a 5-HT_{2C} receptor agonist on nicotine-induced hyperactivity. Intra-VTA injection of the 5-HT_2 receptor agonist Ro 60-0175 decreased cocaine-induced hyperactivity, (Fletcher et al., 2004); however, this effect may be due to stimulation of multiple 5-HT receptor subtypes, including 5-$HT_{2A/B}$ receptors. Although intra-VTA administration of WAY 161503 did not alter locomotor activity, the microinjection site was confirmed as active because intra-VTA administration of the nAch receptor agonist cytisine induced locomotor increasing effects.

The present results indicate that activation of 5-HT autoreceptors may differ in terms of their effects on nicotine-induced hyperactivity. 5-HT_{1A} (presumably somatodendritic autoreceptors) receptor stimulation does not alter nicotine-induced hyperactivity, whereas 5-HT_{1B} (presumably terminal autoreceptors) receptor stimulation may increase this effect. In contrast to activation of 5-HT autoreceptors, activation of the post-synaptic 5-HT_{2C} receptors results in a decrease in nicotine-induced hyperactivity. Our data indicate that this effect may not be mediated by a direct action in the VTA.

Future studies should examine the effects of alternative microinjection sites within the brain on nicotine-induced hyperactivity. For instance, administration of 5-HT_{1A} receptor agonists into the DRN may increase nicotine-induced hyperactivity due to alterations in mesocorticolimbic DA levels. Studies should also address if presynaptic 5-HT_{1A} receptor stimulation differentially affects nicotine-induced hyperactivity compared to action at post-synaptic 5-HT_{1A} receptors. In addition to the mesocorticolimbic system, 5-HT_{2C} receptors and 5-HT_{1B} receptors are also localized in the substantia nigra, ventral pallidum and the hippocampus (areas known to be involved in motor activity). Administration of 5-HT_{2C} or 5-HT_{1B} receptor agonists into areas such as may alter nicotine-induced hyperactivity. Activation of 5-HT_{1B} and 5-HT_{2C} receptors are believed to alter mesocorticolimbic DA levels via action of heteroceptors on GABA neurons (Di Giovanni et al., 2001; Di Matteo et al., 2001; Di Matteo et al., 2002; Johnson et al., 1992; Parsons et al., 1999; Yan and Yan, 2001; Yan et al., 2004); co-administration of GABA receptor ligands may alter the effect of 5-HT_{1B} and 5-HT_{2C} receptor agonist on nicotine-induced hyperactivity. Such future studies may aid in determining the site of action of 5-HT-receptor agonists on nicotine-induced hyperactivity as well as the mechanism responsible for that effect.

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Chapter 6. General discussion

Many studies have attempted to uncover the neural basis of motivation and reward through behavioural testing (e.g. place conditioning, ICSS, drug self-administration) of laboratory animals. Within the brain, 5-HT receptor subtypes may play a role in reward and reinforcement, possibly through modulation of DA in the mesocorticolimbic system. Reinforcing effects of non-selective and selective 5-HT_{1B} and 5-HT_{2C} receptor-related ligands were investigated as activation of these 5-HT receptor subtypes are known to alter DA release in the mesocorticolimbic system. In this thesis, drugs that act at the 5-HT_{1B} or 5-HT_{2C} receptor were assessed on place conditioning and spontaneous locomotor activity. In addition, nicotine-induced hyperactivity was used to examine DA/5-HT interactions. Studies examining the effects of 5-HT receptor ligands on reward and reinforcement may provide insight into the neurochemical abnormalities underlying disorders such as depression and schizophrenia that display impairments in motivation and reward.

Increases in mesocorticolimbic DA activity may be associated with rewarding effects observed in behavioural tests (Blaha and Phillips, 1990; Blanchard et al., 1993; Duvauchelle et al., 2000; Hernandez and Hoebel, 1988; Kiyatkin and Stein, 1995; Pettit and Justice, 1989, 1991; Wise, 2004; You et al., 2001). As 5-HT_{2C} receptor activation decreases DA activity within this system (Di Giovanni et al., 2000; Di Matteo et al., 1999, 2000; Di Matteo et al., 2002; Millan et al., 1998), it was predicted that 5-HT_{2C} receptor stimulation may induce aversive reinforcing effects in the place conditioning test. However, activation of 5-HT_{2C} receptors by the selective 5-HT_{2C} receptor agonist WAY 161503 or the mixed 5-HT receptor agonist TFMPP did not induce place

conditioning when animals were tested in a drug-free state (Chapter 2). To date, no studies have reported the behavioural effects of selective 5-HT_{2C} receptor ligands on place conditioning; however, the non-selective 5-HT_{2C} receptor agonist mCPP did not induce place conditioning effects when animals were tested in a drug-free state (Rocha et al., 1993). The results of the current study are consistent that of Rocha et al. (1993) and indicate that 5-HT_{2C} receptor activation does not induce conditioned reinforcing effects as measured by place conditioning.

It has been reported that some compounds can induce place conditioning effects when animals are tested in a drugged-state (i.e. in the presence of the drug the animal was conditioned with) (Mucha and Iversen, 1984; Nomikos and Spyraki, 1988; Oberling et al., 1993; Olmstead and Franklin, 1997). Therefore, 5-HT_{2C} receptor stimulation was investigated for state-dependent effects in the place conditioning test. This study revealed that 5-HT_{2C} receptor activation induced a state-dependent CPA (Chapter 3). To our knowledge, this is the first report of 5-HT_{2C} receptor activation inducing state-dependent effects in the place conditioning test. It is possible that the observed CPA may be due to a reduction in DA activity within the mesocorticolimbic system following 5-HT_{2C} receptor stimulation; however this may not be sufficient to explain the state-dependent nature of the CPA observed. It was speculated that 5-HT_{2C} receptor stimulation may alter the animals' 'perception' of the conditioning environment and perhaps the animals were not able to recognize the conditioning environment when tested in a drug-free state (Chapter 3).

Environmental cues are known to be important for the acquisition of conditioned drug effects. For example, rats were administered morphine in either the colony room or

another room (distinct from the colony room environment) and then the analgesic effects of morphine were measured using a paw-pressure analgesiometer or the latency which the rats would lick their foot while on a hot-plate in the respective conditioning environment. After eight conditioning sessions, it was determined that rats tested for the analgesic effects of morphine in the environment to which they were conditioned displayed morphine tolerance. However, if the rats were tested in the other environment (i.e. opposite to the one they were conditioned in), no tolerance to the analgesic effects of morphine had developed (Siegel, 1976). Thus, environmental cues in the conditioning environment may be important in order to observe conditioned drug effects. If activation of the 5-HT_{2C} receptor does alter the animals' 'perception' of the environment, place conditioning effects may only be evident when animals are tested in a drugged state. The current study suggests a role for 5-HT_{2C} receptors in state-dependent learning and underscores the need for state-dependent controls when assessing reinforcing drug effects on place conditioning (Chapter 3).

In the first experiment, stimulation of the 5-HT_{2C} receptor did not induce reinforcing effects in the place conditioning test (Chapter 2). Therefore, in order to determine if activation of this receptor induces behavioural reinforcing effects, we examined the effect of WAY 161503 administration in the CTA paradigm. The CTA paradigm was chosen as it is known to be very sensitive to the stimulus properties of drugs (Goudie, 1986). This experiment demonstrated that 5-HT_{2C} receptor stimulation by WAY 161503 was able to induce a CTA to a saccharin solution (Chapter 3). To our knowledge, this is the first report of selective 5-HT_{2C} receptor activation inducing CTA; however, this result is consistent with the mixed 5-HT receptor agonists (with action at

the 5-HT_{2C} receptor) TFMPP and mCPP inducing aversive reinforcing effects in this paradigm (De Vry et al., 2000; Guitton and Dudai, 2004). In the present study, 5-HT_{2C} receptor stimulation induced aversive reinforcing effects on both place conditioning and CTA. However, unlike the observed CPA, the CTA induced by WAY 161503 was not state-dependent. It was speculated that these two behaviours may be mediated by different brain regions and that this may account for the differential effects (i.e. state-dependency) observed (Chapter 3). The current aversive reinforcing effects following 5-HT_{2C} receptor activation are in agreement with the reward-decreasing effects observed following stimulation of this receptor in studies of nicotine, cocaine and ethanol self-administration (Fletcher et al., 2004; Grottick et al., 2001; Tomkins et al., 2002). In general, 5-HT_{2C} receptor agonists induce aversive conditioned reinforcing effects as well as reduce the rewarding properties of drugs of abuse.

In contrast to 5-HT_{2C} receptor activation, 5-HT_{1B} receptor stimulation may increase mesocorticolimbic DA function (Iyer and Bradberry, 1996; O'Dell and Parsons, 2004; Yan and Yan, 2001; Yan et al., 2004). Increases in mesocorticolimbic DA release may be associated with an increase in reward (Blaha and Phillips, 1990; Blanchard et al., 1993; Duvauchelle et al., 2000; Hernandez and Hoebel, 1988; Kiyatkin and Stein, 1995; Pettit and Justice, 1989, 1991; Wise, 2004; You et al., 2001) as well as locomotor hyperactivity (Clarke et al., 1988; Fung and Lau, 1988; Koob, 1992). Thus, administration of the 5-HT_{1B} receptor-related agonists RU 24969 and CP 94253 were predicted to induce positive reinforcing effects on place conditioning and increase locomotor activity. Selective stimulation of the 5-HT_{1B} receptor by CP 94253 did not alter spontaneous locomotor activity (Chapter 4). The current result of 5-HT_{1B} receptor

stimulation by CP 94253 is consistent with that of Koe et al. (1992); CP 94253 did not alter locomotor activity except at a high dose (32.0 mg/kg) (Koe et al., 1992). Although activation of 5-HT_{1B} receptors may increase DA transmission within the mesocorticolimbic system (Yan and Yan, 2001; Yan et al., 2004), no changes in spontaneous locomotor activity were observed.

The mixed 5-HT receptor agonist RU 24969 induced locomotor hyperactivity, an effect which was blocked by the selective 5-HT_{1A} receptor antagonist WAY 100635 and attenuated by the 5-$HT_{1B/D}$ receptor antagonist GR 127935. Blockade of the 5-HT_{2C} receptor by SB 242084 did not affect the locomotor stimulant effects induced by RU 24969 (Chapter 4). RU 24969-induced locomotor hyperactivity observed in the present study is consistent with previous reports (Carli et al., 1988; Cheetham and Heal, 1993; Goodwin and Green, 1985; Kalkman, 1995; Oberlander et al., 1987; Rempel et al., 1993); however, there has been debate over which 5-HT receptor subtype is responsible for this effect. Discrepancy in the literature may be due to methodology differences between laboratories such as the species or locomotor apparatus/measurement used. In rats, RU 24969-induced hyperactivity was blocked by the 5-HT_{1A} receptor antagonist WAY 100635 and the partial 5-HT_{1A} receptor antagonist SDZ 216-525 but not the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (Kalkman, 1995). In contrast, Chaouloff et al. (1999) attributed RU 24969-induced hyperactivity to the 5-HT_{1B} receptor as GR 127935 was reported to reduce this behaviour; other studies have also supported a role for 5-HT_{1B} receptors mediating RU 24969-induced hyperactivity (Cheetham and Heal, 1993; Rempel et al., 1993). Furthermore, O'Neill and Parameswaran (1997) suggested that RU 24969-induced hyperactivity may be mediated by activation of both the 5-HT_{1A} and 5-HT_{1B}

receptors. Results from the current study are in agreement with Kalkman et al. (1996) indicating that the locomotor stimulant effects of RU 24969 may be mediated via stimulation of the 5-HT_{1A} receptor. This result is also in agreement with previous reports which demonstrated that stimulation of 5-HT_{1A} receptors may increase locomotor activity (Bjork et al., 1992; Kalkman and Soar, 1990), however this result has not consistently been replicated (Hillegaart et al., 1989; Mittman and Geyer, 1989). In addition, this study also suggests that activation of the 5-HT_{1B} receptor may also contribute to the locomotor stimulant effects of RU 24969 as suggested by O'Neill and Parameswaran (1997). Attenuation of RU 24969-induced locomotor hyperactivity may not have been observed by Kalkman et al. (1996) as a lower dose (1.0 mg/kg) of GR 127935 was used compared to those used by O'Neill and Parameswaran (1997), Chaouloff et al. (1999) and the current study. Although RU 24969 is generally cited as a 5-HT_{1B} receptor agonist, the effect on locomotor activity observed in the current study is not consistent with that following selective stimulation of the 5-HT_{1B} receptor. Therefore, the classification of RU 24969 should be changed to reflect the fact that 5-HT_{1A} receptor stimulation may be involved in RU 24969-induced behaviours (i.e. locomotor activity).

Administration of RU 24969 or CP 94253 induced a CPA in the current study (Chapter 4). The observed CPA following CP 94253 administration in the current experiments is in agreement with Cervo et al. (2002); however, this is the first study to report aversive reinforcing properties of RU 24969 on place conditioning. This result is of interest as compounds that induce locomotor stimulant effects such as AMPH, nicotine and cocaine also induce rewarding effects in the place conditioning test. Previously, 5-HT_{1B} receptor ligands have been reported to induce anxiogenic effects in behavioural

tests such as the elevated plus maze (Benjamin et al., 1990; Lin and Parsons, 2002; Pellow et al., 1987); anxiety-inducing effects following activation of this receptor have not been consistently reported (Bell et al., 1995; Chojnacka-Wojcik et al., 2005). In addition, compounds which are anxiogenic such as yohimbine, picrotoxin, BIBP3226 (neuropeptide Y antagonist) and bicuculline have been reported to induce a CPA (File, 1986; Kask et al., 1999; Thielen and Shekhar, 2002). Thus, the CPA induced by CP 94253 or RU 24969 administration may be due to the induction of anxiogenic effects following administration of these compounds. Further investigation will be needed to determine how activation of the 5-HT_{1B} receptor induces aversive reinforcing effects in the place conditioning test.

In general, activation of 5-HT_{1A} receptors by systemic 8-OHDPAT administration induces positive reinforcing effects as measured by the induction of a CPP (Fletcher et al., 1993; Papp and Willner, 1991; Shippenberg, 1991). However, systemic 8-OHDPAT (1.0 mg/kg) induced a CPA when the animals were conditioned on the side that was initially preferred during pre-conditioning (biased design) (Papp and Willner, 1991). The CPA induced by RU 24969 in the current study was also observed in a place conditioning biased design, similar to that observed following 8-OHDPAT administration. However, in contrast to the affect of 8-OHDPAT on place conditioning, lower doses of RU 24969 did not induce a CPP in either a biased or unbiased place conditioning design. The effects on place conditioning displayed by RU 24969 in the current study are not consistent with activation of either the 5-HT_{1A} or 5-HT_{1B} receptor alone. Therefore, it is possible that the CPA induced by RU 24969 may be mediated by activation of multiple

5-HT receptor subtypes; however, further examination will be needed to determine which 5-HT receptor subtype(s) is responsible for this behaviour.

In contrast to the CPA induced following 5-HT_{2C} receptor stimulation, the CPA induced by RU 24969 and CP 94253 was observed when the animals were tested in a drug-free state. The differential nature (i.e. state-dependency) of the CPAs induced by the 5-HT receptor ligands may be due to activation of different 5-HT receptor subtypes. Drugs such as LSD that alter perception in humans act primarily on receptors in the 5-HT_2 receptor family (Kadan et al., 1984). Therefore, conditioned reinforcing effects may be evident when animals are tested in a drug-free state following conditioning with a 5-$HT_{1A/B}$ receptor agonist as ligands at these receptors may not alter the animals' 'perception' of the conditioning environment.

Experiments in the current study also re-affirmed previous reports in the literature related to 5-HT_{2C} receptor ligands and locomotor activity. Selective stimulation of the 5-HT_{2C} receptor by WAY 161503 dose-dependently decreased spontaneous locomotor activity. This result is in agreement with previous studies using partial 5-HT_{2C} receptor agonists such as mCPP and Ro 60-0175 (Gleason et al., 2001; Higgins et al., 2001; Kennett et al., 2000; Kennett et al., 1997; Martin et al., 2002) and indicates a role for 5-HT_{2C} receptors in mediating locomotor activity. In addition, previous studies have demonstrated that selective blockade of the 5-HT_{2C} receptor by SB 242084 did not significantly alter locomotor activity (Fletcher et al., 2002; Kennett et al., 1997; Martin et al., 2002); this result was replicated in the present study (Chapter 2).

In this thesis, TFMPP- induced locomotor hypoactivity was reversed by pretreatment with the selective 5-HT_{2C} receptor antagonist SB 242084 but not the

selective 5-HT_{1A} or 5-$HT_{1B/D}$ receptor antagonists WAY 100635 or GR 127935 (Chapter 2). Lucki et al. (1989) suggested that the decrease in locomotor activity induced by TFMPP may be due to activation of the 5-HT_{2C} receptor, but, due to the previous lack of selective 5-HT_{2C} receptor antagonists this hypothesis could not be tested. The results of the current study confirm the original suggestion by Lucki et al. (1989) indicating that the locomotor suppressant effects of TFMPP are mediated by stimulation of the 5-HT_{2C} receptor. Compounds which act as agonists at the 5-HT_{2C} receptor are known to decrease the firing of DA neurons within the VTA as well as decrease DA transmission in terminal areas of the mesocorticolimbic system (Di Giovanni et al., 2000; Di Matteo et al., 1999, 2000; Di Matteo et al., 2002; Millan et al., 1998). As increases in mesocorticolimbic DA release may be associated with an increase in locomotor activity (Clarke et al., 1988; Fung and Lau, 1988; Koob, 1992), systemic administration of a 5-HT_{2C} agonist may reduce locomotor activity due to an influence on mesocorticolimbic DA release (Prisco et al., 1994). This study also demonstrates that although TFMPP and RU 24969 demonstrate a similar receptor profile (i.e. 5-$HT_{1A/B/2C}$ receptor agonist), differential results from behavioural studies may be due to a preferential action at different 5-HT receptor subtypes.

The present study also investigated the effects of 5-HT_{1B} or 5-HT_{2C} receptor activation on nicotine-induced hyperactivity. Nicotine-induced hyperactivity is known to increase extracellular DA concentrations in the mesocorticolimbic system (Benwell and Balfour, 1992; Benwell et al., 1995; Clarke et al., 1988; Damsma et al., 1989; Imperato et al., 1986; Nisell et al., 1994; Nisell et al., 1996; Olausson et al., 2001; Rahman et al., 2003). As 5-HT_{1B} and 5-HT_{2C} receptors are known to alter DA release in this system (Di

Giovanni et al., 2000; Di Matteo et al., 1999, 2000; Di Matteo et al., 2002; Millan et al., 1998; Yan and Yan, 2001; Yan et al., 2004), 5-HT/DA interactions were investigated using nicotine-induced hyperactivity. It was hypothesized that activation of 5-HT_{2C} receptors would attenuate whereas stimulation of 5-HT_{1B} receptors would potentiate nicotine-induced hyperactivity. Following systemic administration of the 5-HT_{2C} receptor agonist WAY 161503, nicotine-induced hyperactivity was decreased; 5-HT_{1B} receptor activation by CP 94253 potentiated this behaviour (Chapter 5). This is consistent with the proposed role of 5-HT_{2C} receptor stimulation decreasing and 5-HT_{1B} receptor stimulation increasing DA function within the mesocorticolimbic system. The result of systemic 5-HT_{2C} receptor activation decreasing nicotine-induced hyperactivity is consistent with Grottick et al. (2001); however to our knowledge this is the first report of systemic 5-HT_{1B} receptor activation potentiating the locomotor stimulant effect of nicotine. As systemic 5-HT_{2C} receptor activation decreases both the locomotor stimulatory effects of nicotine as well as nicotine self-administration, agonists which act at this receptor may be useful in treating smoking cessation (Grottick et al., 2001).

Alterations of nicotine-induced hyperactivity following systemic administration of the 5-HT_{1B} or 5-HT_{2C} receptor agonists may be due to stimulation of these 5-HT receptor subtypes in multiple brain areas. 5-HT_{1B} (Yan and Yan, 2001; Yan et al., 2004) or 5-HT_{2C} receptor (Di Giovanni et al., 2000; Di Matteo et al., 1999, 2000; Gobert et al., 2000) agonists as well and nicotine (Mereu et al., 1987; Nisell et al., 1996; Rahman et al., 2003) have previously been shown to alter DA function in the VTA. Therefore, the current study investigated whether intra-VTA microinjections of CP 94253 or WAY 161503 would affect nicotine-induced hyperactivity. It was hypothesized that intra-VTA

infusion of CP 94253 may potentiate and WAY 161503 infusion may attenuate nicotine-induced hyperactivity. However, intra-VTA administration of the 5-HT_{1B} or 5-HT_{2C} receptor agonist failed to alter nicotine-induced hyperactivity. These results indicate that stimulation of 5-HT_{1B} or 5-HT_{2C} receptors in the VTA may not responsible for mediating the affect of systemic 5-HT_{1B} or 5-HT_{2C} receptor agonist administration on nicotine-induced hyperactivity.

It has been speculated that decreases in DA release in the mesocorticolimbic system may be responsible for the locomotor suppressant effect observed following 5-HT_{2C} receptor stimulation. However, intra-VTA infusion of the 5-HT_2 receptor agonist Ro 60-0175 (Fletcher et al., 2004) or 5-HT_{2C} receptor blockade following intra-VTA or intra-NAS administration of RS 102221 (McMahon et al., 2001) failed to alter locomotor activity. Thus, regions outside of the mesocorticolimbic system should be investigated to determine which brain areas are responsible for the effects of 5-HT_{1B} or 5-HT_{2C} receptor activation on locomotor activity. In addition to the mesocorticolimbic system, 5-HT_{2C} receptors are also localized to the substantia nigra and the ventral pallidum is densely populated with 5-HT_{1B} receptors (areas both involved in motor activity). It is likely that these areas may be involved in the effects of 5-HT_{1B} or 5-HT_{2C} receptor activation on locomotor activity.

Limitations

Intra cranial microinjections have been used to determine specific brain regions involved in various behaviours; however, this technique does have some limitations (see Carvey et al. 1994; McBride et al. 1999). When compounds are infused into the brain,

diffusion usually occurs in a circular gradient. However, nonspecific drug exposure to nearby structures may occur as anatomical structures generally are not circular in shape. In the present experiments, drugs were infused in a volume of 0.5 μL in an attempt to limit diffusion outside of the VTA. Upon experimental completion, microinjection sites were confirmed histologically.

The main limitations of place conditioning are: results are subject to interpretation based on novelty seeking and results can be difficult to interpret if the animals prefer one side of the place conditioning apparatus during the pre-conditioning phase. In addition experimental groups in the place conditioning test is a between subjects factor. Therefore, examining large dose response curves can be labor intensive and many animals may be required (Bardo and Bevins, 2000; Swerdlow et al., 1989). However, despite these limitations, place conditioning remains a widely used behavioural parardigm to assess reinforcing drug effects in laboratory animals.

Conclusions and future studies

5-HT receptor-related compounds may influence reward and reinforcement by altering DA neuronal activity in the mesocorticolimbic system via GABAergic interneurons in the VTA. Future experiments investigating the effects of 5-HT_{1B} or 5-HT_{2C} receptors on reward should employ microdialysis to measure DA release in this system following administration of 5-HT_{1B} or 5-HT_{2C} receptor ligands in behavioural tests used to measure reward such as place conditioning and ICSS. In addition, it has been hypothesized that 5-HT_{1B} and 5-HT_{2C} receptor ligands located in the VTA may alter mesocorticolimbic DA activity by mediating GABAergic inhibition on DA neurons (Di

Giovanni et al., 2000; Di Giovanni et al., 2001; Di Matteo et al., 2000; Di Matteo et al., 2001; Johnson et al., 1992; Parsons et al., 1999; Yan et al., 2004). Therefore, co-administration of GABAergic compounds should be tested in combination with 5-HT_{1B} or 5-HT_{2C} receptor-related drugs to assess the influence of these compounds on 5-$HT_{1B/2C}$- induced behaviours. These experiments would be complementary to the existing electrophysiology literature relating 5-HT_{1B} or 5-HT_{2C} receptor stimulation to mesocorticolimbic DA release and help clarify the neural mechanism by which compounds that act at these 5-HT receptor subtypes induce behavioural reinforcing effects.

A recent study by Zhang et al. (2005) indicated that salvinorin A (an hallucinogen derived from the plant Salvia divinorum) induced a CPA as well as decreased locomotor activity during place conditioning sessions. Due to the state-dependent nature of the CPA induced by the 5-HT_{2C} receptor agonist WAY 161503, a conditioned locomotor activity experiment should also be undertaken. It was proposed that the CPA induced by WAY 161503 may be due to effects on sensorimotor integration (Chapter 3). Such an experiment would address if differential effects on locomotor activity are induced if animals are tested in a drug-free or drugged state following conditioning to the locomotor activity boxes. Thus, a conditioned locomotor experiment would aid in determining if activation of the 5-HT_{2C} receptor by WAY 161503 alters the animals' sensorimotor integration.

Furthermore, place conditioning experiments should be repeated using RU 24969 with selective 5-HT_{1A} and 5-HT_{1B} receptor antagonists to determine which 5-HT receptor

subtype(s) is responsible for the aversive reinforcing effects of this compound in the place conditioning test.

The CPP induced by AMPH treatment in some studies was extinguished by post-conditioned day 2 (see Figure 3.2) as measured over the total 15 minute test period. Future studies should examine the local time course effects of AMPH during post-conditioning testing days (i.e. time spent in drug-paired compartment during 5 min intervals). This would aid in determining if the place conditioning effects of AMPH are still present during the initial exposure to the apparatus during retention testing sessions.

In the current study, intra-VTA administration of 5-HT_{1B} or 5-HT_{2C} receptor agonists failed to alter nicotine-induced hyperactivity. However, as nicotine-induced hyperactivity is known to be mediated by DA activity in the mesocorticolimbic system (Benwell and Balfour, 1992; Benwell et al., 1995; Damsma et al., 1989; Imperato et al., 1986; Nisell et al., 1994; Nisell et al., 1996; Olausson et al., 2001), future studies should examine the effects of 5-HT_{1B} or 5-HT_{2C} receptor ligand administration on this behaviour following microinjections into areas such as the NAS or PFC. Systemic 5-HT_{1B} or 5-HT_{2C} receptor agonists alter nicotine-induced hyperactivity. Therefore, systemic administration of ligands which act at these 5-HT receptor subtypes should be examined on nicotine-induced behaviours in other tests such as place conditioning and ICSS.

Such further behavioural experiments will help to clarify the neural mechanism underlying the reinforcing effects induced by 5-HT_{1B} and 5-HT_{2C} receptor ligands. In addition, they may aid in further characterization of 5-HT/DA interactions in the mesocorticolimbic system. Dysfunction of multiple 5-HT and DA receptor subtypes have been implicated in the neurochemical abnormalities of disorders that display

impairments in motivation and reward. Therefore, behavioural investigation of 5-HT/DA interactions within the mesocorticolimbic system in the context of reward/reinforcement may lead to the development of better therapeutic drugs to treat these disorders.

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